

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

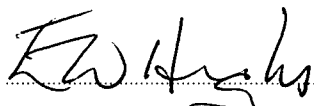
Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.



Elizabeth W. Hughes, B.A., M.Sc.,
Study Director,
Huntingdon Life Sciences Ltd.

14 July 1997
Date



Allen R. Sutton,
Sponsor's Representative.

25 July 1997
Date



~~CONFIDENTIAL~~

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IMF 7/962745

N-(n-butyl) thiophosphoric triamide (NBPT)

**TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS
INCORPORATING A NEUROTOXICITY SCREEN**

Volume 1

Sponsor

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Report issued 14 July 1997



QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to Management, Huntingdon Life Sciences are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	28 May 1996
Pre-experimental Period	10 June 1996	12 June 1996
Experimental Period	17/20 June 1996	21 June 1996
	3 July 1996	3 July 1996
	9 & 11 September 1996	12 September 1996
	27 September 1996	30 September 1996

Date of reporting audit findings to the
Study Director and Management

26 March 1997



Rod Scammell,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

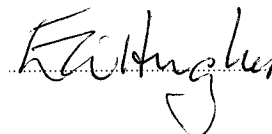
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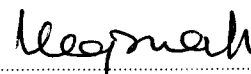
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SUMMARY

In this experiment, the toxicity of (N-(n-butyl) thiophosphoric triamide (NBPT), an organophosphorus compound and a urease inhibitor, was assessed in three groups of 15 male and 15 female Crl:CD BR rats which were given NBPT by dietary admixture at levels of 200, 1000 and 5000 ppm for 13 weeks. Dietary inclusion levels remained constant throughout the study. The overall achieved mean intakes in terms of mg/kg/day for main animals were 14.7, 74 and 377 for males and 17.4, 88 and 445 for females. A similar sized group was given untreated diet and acted as controls. Ten animals/sex/group were designated as Main group animals with the remaining five animals/sex/group designated as Satellite animals.

All animals were monitored for clinical signs, bodyweights and food consumption. Water consumption was measured during Week 12. The eyes of all control animals and animals treated at 5000 ppm were subjected to an ophthalmoscopic examination in Week 13. Laboratory investigations (haematology and clinical chemistry) were performed on all Main group animals in Weeks 5 and 13.

During the pre-dose period and Weeks 4, 8 and 13, 5 males and 5 females from the Main group and all the Satellite animals were screened for neurotoxicity (*ie* scored on a functional observational battery and were quantitatively assessed for locomotor activity). At Week 14 all the Satellite animals were killed using whole body perfusion and selected tissues of the nervous system were then examined histopathologically. At Week 14 all Main group animals were killed, a *post mortem* examination performed and organ weights were recorded for selected tissues. Histopathology was performed on indicated tissues.

Clinical signs and mortalities

One female treated at 5000 ppm was considered to be affected by treatment showing signs of unsteady gait, hunched posture and piloerection. These signs were exhibited during Weeks 3 to 10.

There were no mortalities associated with treatment.

Bodyweights

Cumulative gains were statistically significantly lower than controls among males and females treated at 5000 ppm from the commencement of treatment to Week 4. There was a suggestion among males at 5000 ppm of a slight reduction in weight gains during Weeks 8 to 12.

Food consumption

Cumulative intakes (Weeks 1 to 12) among males treated at 5000 ppm were statistically significantly lower than controls.

Efficiency of food utilisation

During the first 3 weeks of treatment, food utilisation was clearly impaired among males and females treated at 5000 ppm.

Water consumption

There was no effect of treatment.

Neurotoxicity screening

There were transitory changes in behaviour during Week 4 which were characterised by decreased grip strength for all animals treated at 5000 ppm and a low incidence of hunched posture in females at 5000 ppm. With continuing exposure, these changes disappeared.

Ophthalmoscopic examination

There was no effect of treatment.

Haematology

During Week 5, white blood cell counts were statistically significantly lower among males treated at 5000 ppm due to increased lymphocyte counts.

Among females treated at 5000 ppm, platelet counts were statistically significantly increased at Week 5 and Week 13.

Biochemistry

Statistically significant differences were observed in the following parameters at 5000 ppm:

GPT decreased among males in Week 5 and among females in Weeks 5 and 13; GOT decreased among males in Weeks 5 and 13, and among females in Week 5; AP decreased among males in Week 13 and among females in Week 5; phosphorus levels decreased among males in Weeks 5 and 13 and among females in Week 13

At 1000 ppm the following parameters were statistically significantly different:

GOT decreased among males in Week 5; AP decreased among males in Week 13; phosphorus levels decreased among females in Week 13

At 200 ppm the following parameters were statistically significantly different:

Phosphorus levels decreased among females in Week 13

Organ weights

Liver weights when adjusted for bodyweights were statistically significantly increased among males treated at 5000 ppm when compared with controls. Uterus weights of females treated at 1000 or 5000 ppm were statistically significantly increased compared with controls.

Macroscopic pathology

An increased incidence of fluid distension of the uterus was observed among females treated at 200, 1000 or 5000 ppm compared with controls.

Histopathology

Among males, a statistically significantly increased incidence of minimal centrilobular hepatocyte hypertrophy was observed in male rats 5000 ppm. Among female rats, at 5000 ppm, an increase in the incidence of foci of mineralisation in the kidney was observed. There was also a treatment related increase in the incidence of luminal dilatation of the uterus for females treated at all dosages of NBPT.

Neuropathology provided no evidence of neurotoxicity.

Conclusion

Treatment with NBPT for thirteen weeks at dietary inclusion levels of 0, 200, 1000 and 5000 ppm was associated with a range of effects.

At 5000 ppm, treatment was associated with some reactions that showed subsequent recovery (clinical signs, bodyweight gains, food utilisation, and responses on the functional observational battery) as well as some effects that continued throughout treatment (cumulative food consumption and haematology and biochemistry parameters). Organ weight and histopathological changes were also observed.

At 1000 ppm, there were limited effects on some biochemistry parameters and among females, an increased incidence of luminal dilatation of the uterus.

The no observed effect level (NOEL) was established in the males as being 200 ppm. However, it was not possible to demonstrate a NOEL among females as a slightly lower phosphorus level and a low incidence of luminal dilatation of the uterus was observed. The toxicological significance of these findings, if any, cannot be determined at this time.



INTRODUCTION

The object of this study, performed at the Huntingdon Life Sciences, Huntingdon, England, was to assess the toxicity and the potential neurotoxicity of the test substance, NBPT, to rats by dietary administration over a period of 13 weeks.

The neurotoxicity component was designed in accordance with EPA FIFRA Pesticide Assessment Guidelines, Subdivision F, Addendum 10, Neurotoxicity, published March 1991, which is also applicable to neurotoxicity testing of industrial chemicals for TSCA. Otherwise, this study was designed in accordance with EPA TSCA Test Guidelines, 40 CFR Part 798 - Health Effects Testing Guidelines, September 27 1985, 798.2650, Oral Toxicity and Subsequent Revision, May 20 1987.

The treatment levels employed 0 (control), 200, 1000 and 5000 ppm were selected by the Sponsor based on a two week palatability/toxicity study performed in these laboratories (IMF/6).

The rat was the species of choice due to regulatory requirements and the Crl:CD BR strain was chosen due to availability of background data.

The route of exposure, dietary, was selected since the oral route is a potential route of human exposure to the test material.

RELEVANT STUDY DATES

Protocol approval by:

Study Director	22 May 1996
Huntingdon Management	22 May 1996
Sponsor	28 May 1996

Animal arrival at Huntingdon: 29 May 1996

Treatment commenced: 10 June 1996

Neurotoxicity screening:

Pre-dose	4 - 7 June 1996
Week 4	1 - 4 July 1996
Week 8	29 July - 1 August 1996
Week 13	2 - 5 September 1996

Water consumption:

Week 12 26 August - 2 September 1996

Ophthalmoscopic examination:

Pre-dose	3 June 1996
Week 13	
Females	3 September 1996
Males	4 September 1996

Haematology/biochemistry:

Week 5	9 July 1996
Week 13	6 September 1996

Terminal kill: 9 - 12 September 1996

TEST SUBSTANCE

Test substance:	N-(n-butyl) thiophosphoric triamide (NBPT)
Action:	Organophosphorus compound, a urease inhibitor
Description of substance:	Light tan waxy solid
Storage conditions:	Ca +4°C in the dark
Batch No.:	8124-165
Purity:	89.5% - 89.6% (see certificate of analysis)
Date of receipt at Huntingdon:	21 February 1996
Supplier:	Albermarle Corp, PO Box 14799, Baton Rouge, LA 70898, USA
Stability of test substance:	December 1996

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

A total of 158 Crl:CD BR rats (79 males and 79 females) approximately 35 days old and within a weight range of 15 g for each sex, were received from Charles River Breeding Laboratories, Manston Road, Margate, Kent, England.

Animals selected for the study were at least 6 weeks and their bodyweights were in the range 240 g to 283 g for males and 162 g to 207 g for females.

On arrival, five males and five females selected at random were used for health check purposes. These animals were killed within 24 hours after arrival at Huntingdon and subjected to routine macroscopic examination. No abnormalities were detected. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further.

The remaining rats were placed at random in suspended cages with wire mesh floors so that each cage contained one rat. Each cage measured 21 cm wide, 26 cm deep and 27 cm high.

Animal room temperature and relative humidity controls were maintained at $21 \pm 2^{\circ}\text{C}$ and $55 \pm 10\%$ respectively. Recorded values were generally within $21^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and $51\% \pm 9\%$ respectively. Permanent weekly recordings of these parameters were made by a Cambridge recorder and these are archived with all other raw data for this study. Lighting was controlled to give 12 hours continuous light and 12 hours continuous dark per 24 hours.

All rats had free access to tap water and ground SDS Rat and Mouse No. 1 maintenance diet except as noted under **LABORATORY INVESTIGATIONS**. There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test substance could reasonably be expected to be present in the diet, or the tap water, both of which were routinely subjected to regular chemical analysis, results of which are lodged in the Huntingdon Life Sciences Archives.

After an acclimatisation period of 5 days, each animal was weighed and the required number of animals were selected by discarding those animals furthest from the mean bodyweight. The remaining animals were then randomly assigned to groups, stratified by bodyweight, in such a way that the initial group means were approximately equal. The appropriate numbers of cages were then allocated to each treatment group.

A further period of acclimatisation of 7 days was allowed between allocation of animals to groups and the commencement of treatment. During this period a review of animal health was undertaken by a veterinary officer. The spare animals were retained during this acclimatisation period to replace any rat showing signs of ill health. The pre-dose ophthalmoscopy was also performed during this period. As a result, some of these spare rats were used to replace animals rejected due to the ophthalmoscopic examination (see section **Ophthalmic examination**). Within 48 hours of the commencement of treatment all animals that were excess to requirements were discarded with no further investigations performed.

Throughout the study the animals were housed in the Department of Rodent Toxicology, Barriered Rodent Building No. 1BRB, Room 34.

ANIMAL IDENTIFICATION

Group	Rat numbers			
	Main groups		Satellite groups	
	♂	♀	♂	♀
1	1 - 10	41 - 50	81 - 85	101 - 105
2	11 - 20	51 - 60	86 - 90	106 - 110
3	21 - 30	61 - 70	91 - 95	111 - 115
4	31 - 40	71 - 80	96 - 100	116 - 120
Health check	121 - 125	126 - 130		

The rats were housed individually. Each cage was identified by a coloured label according to group and each label was uniquely numbered with cage and study number. The cage number was tattooed on the leg of each rat in the cage. The cages constituting each group were dispersed in batteries so that possible environmental influences arising from their spatial distribution were equilibrated, as far as possible, for all treatments. In order to accommodate the performance of neurobehavioural screening, animals were assigned to one of four batches:

Batch	Group/animal no.			
	1	2	3	4
Males				
1	6 - 10	16 - 20	26 - 30	36 - 40
2	81 - 85	86 - 90	91 - 95	96 - 100
Females				
3	46 - 50	56 - 60	66 - 70	76 - 80
4	101 - 105	106 - 110	111 - 115	116 - 120

PREPARATION OF FORMULATIONS

The particle size of the test material was reduced prior to weighing by use of a coffee grinder, after particle size reduction by hand if necessary. The test material was then accurately weighed out and plain diet was added, with stirring, to the ground material and the mixture was then ground using a coffee grinder. Dependent upon the concentration, a further amount of diet was added and the mixture ground again using a coffee grinder and diluted to the premix weight with further quantities of plain diet. Mixing of the premix was achieved using a Turbula mixer for a minimum of 5 minutes.

The dietary concentrations required for feeding were made by diluting 2000 g of the appropriate premix to the weight of the diet mix with plain diet, mixing being achieved using a Turbula mixer for a minimum of 5 minutes.

Each diet mix was made from an individual premix. Diets were frozen until used.

Group/colour code	Dosage (ppm)
1: White	Control
2: Yellow	200
3: Green	1000
4: Red	5000

DIETARY SAMPLING AND ANALYSIS

Prior to the start of treatment the proposed diet mixing procedures were checked by chemical analysis of trial diets to confirm that the proposed procedures produced homogenous diet, that the accuracy of mixing was acceptable and that the concentration of test substance in the diet remained unchanged between preparation and administration.

Samples of diets prepared for Week 1 and for Week 11 were also analysed to check the accuracy of preparation.

Chemical analysis was carried out by Huntingdon Department of Analytical Chemistry (see **FORMULATION REPORT**).

ADMINISTRATION OF FORMULATIONS

The test substance, NBPT, was administered by admixture with the diet. Control animals received normal untreated diet.

The concentrations of test substance in the diet of treated groups remained constant throughout the study.

Animals were fed fresh diet on a daily basis with fresh diet being fed in the afternoon. On the two occasions when animals were starved prior to clinical pathology examinations, the diet was fed on the morning following completion of the blood sample procedures.

DURATION OF TREATMENT

Following a total acclimatisation period of 12 days, treatment continued until completion of study at 13 weeks.

OBSERVATIONS AND MEASUREMENTS

Except where indicated, observations apply to Main and Satellite group animals.

Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit as well as to the group observations and examinations outlined in this procedure, were recorded in the Study Day Book.

The following observations were made during the course of the study:

Clinical signs and mortality

Individual animals were observed and palpated at least once daily for any signs of behavioural changes, reaction to treatment or ill health. These examinations were performed on each weekday, at suitable intervals during the day, for the first 4 weeks of the study and were not performed on Saturdays and Sundays. After 4 weeks of treatment, these examinations were performed once weekly.

Dated and signed records of appearance, change and disappearance of clinical signs were maintained on clinical history sheets for individual animals.

Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals. On Saturdays and Sundays and Public Holidays a similar procedure was followed except that the final checks were carried out at approximately mid-day.

There was a single mortality on this study following blood sampling in Week 5. A detailed macroscopic examination was performed and where practicable a full spectrum of tissue samples were preserved and examined.

Bodyweight

The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment, and once a week thereafter.

Animals designated for the functional observational battery were also weighed on each occasion of testing.

Food consumption

The quantity of food consumed by each rat was recorded on a weekly basis for Week -1, the week prior to the start of treatment. Following the commencement of treatment, consumption was recorded on a daily basis. For the purposes of reporting, intake has been presented on a weekly basis.

Food intake per rat was calculated using the amount of food given to and left by each rat during each week using the following formula:

$$\text{Food consumption (g/rat/week)} = \frac{\text{Total food given} - \text{Total food left}}{\text{Number of animal days} *}$$

The results using this formula (present in Appendix 2) were subject to rounding to the nearest whole number.

* The term 'animal day' counts one animal day for each animal alive for a whole day

Efficiency of food utilisation

Efficiency of food utilisation (food conversion ratios) were calculated, where possible, on a weekly basis from Week 1 to 13 from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. The following formula was used:

$$\text{Food conversion ratio} = \frac{\text{Food consumed}}{\text{Bodyweight gain}}$$

The 'food consumed' was calculated as indicated in the **Food consumption** section and was not a mean of the individual cage means. The 'bodyweight gain' was calculated from the gain of each animal and used the mean gain in the formula.

Intake of test substance

At weekly intervals, the group mean achieved intake of test substance (mg/kg/day) was calculated from the group mean bodyweight and food consumption and the dietary inclusion levels of the test material. The following formula was used:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Food consumed (g/rat/week)} \times \text{ppm of test substance}}{\text{Mid-week bodyweight (g/rat)} \times 7 \text{ (days/week)}}$$

The 'food consumed' was calculated as indicated in the **Food consumption** section and was not a mean of the individual cage means. The mid-week bodyweight was calculated from each individual animal and used the mid-week bodyweight in the formula.

Water consumption

Daily monitoring by visual appraisal of the water bottles was maintained throughout the study.

Water consumption was measured accurately, by weight, over daily periods during Week 12 for all rats in all groups.

Ophthalmic examination

Before treatment commenced, the eyes of all animals were examined by means of a Keeler indirect ophthalmoscope. During Week 13 the eyes of all animals in Groups 1 and 4 were examined.

Prior to examination, the pupils of each animal were dilated using a Tropicamide ophthalmic solution.

The ophthalmoscopic examination performed prior to the commencement of treatment resulted in the rejection of one male and 5 female rats:

- one male with right eye prominent hyaloid remnant and small circular opacity;
- one female with right eye a patch of hyper-reflectivity in fundus;
- one female with bilateral retinal disruption around disc;
- one female with left eye intravitreal haemorrhage;
- one female with right eye posterior capsular polar opacity;
- one female with right eye pupil undilated after re-dropping with Mydriacil.

Neurotoxicity screening (Main and Satellite groups)

The functional observational battery and motor activity test were performed on a total of 10 animals/sex/group (5 Main group animals/sex/group and 5 Satellite animals/sex/group).

During the study, a functional observational battery and motor activity were performed at approximately the same time of day, before initiation of treatment and during the 4th, 8th and 13th week of treatment. Not all rats were tested in one day, but time of testing was balanced across the groups. Observations made during the treatment period were made in the afternoon. In addition, observations in Week 13 were performed prior to any laboratory investigations.

The functional observational battery is detailed below:

The battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set was performed when initially handling the animal. The third set of observations was performed in the test arena and the fourth set comprised handling/specific testing of the animal. All these observations were made with the observer blind to the treatment condition of the animal.

Home cage observations:

- Posture in the cage
- Presence of convulsions, tremors, twitches
- Presence of spontaneous vocalisations
- Palpebral closure

Observations in the hand:

- Ease of removing the animal from the cage
- Ease of handling the animal
- Salivation/lacrimation
- Palpebral closure
- Exophthalmus
- Piloerection
- Vocalisation on handling

Observation in the arena:

- Occurrence of convulsions, tremors, twitches
- Level of activity in the arena (number of squares entered counted)
- Level of arousal
- Rearing count
- Grooming
- Assessment of gait
- Record presences of faecal boluses, urine

Manipulations:

Approach response
 Touch response
 Startle response
 Righting reflex
 Tail pinch response
 Pupil reflex
 Grip strength (fore and hindlimb)
 Landing foot splay
 Body temperature (°C)
 Bodyweight (g)

At any point during the observations, additional comments were made as free text where considered appropriate.

Motor activity was performed and monitored using a Coulbourn Infra-Red Activity Monitoring System (system supplied by Coulbourn Instruments, Lehigh Valley, PA, USA).

This system uses an infra-red detector to monitor activity. The following categories of activity are recorded: the time spent in no movement, locomotor, and non-locomotor activity. The number of occurrences (events) of each category is also recorded. For reporting this data, only the time spent in locomotor activity was presented.

For testing, designated animals were placed singly into observation cages. Once all animals had been placed into the cages, the test session programme was started. The test session for each animal was 1 hour. Data was collected every 2 minutes and written onto a floppy disk.

The functional observational battery was performed in Room 17 and the motor activity monitoring was performed in Room 16.

LABORATORY INVESTIGATIONS (Main groups)

During Weeks 5 and 13, samples of blood were withdrawn, under light anaesthetic, from the orbital sinus of all rats from each group.

The blood samples collected were divided into tubes as follows:

EDTA anticoagulant for haematological investigations
 Citrate anticoagulant for coagulation tests
 Heparin anticoagulant for biochemical tests

Food was removed overnight from animals sampled for laboratory investigations.

The estimations performed on blood samples have been listed below and overleaf, together with an abbreviated title (for use in Appendices and Tables), the methods and the units of measurement applicable at the time.

Haematology

Units

The following estimations were performed using a Bayer-Technicon H1E haematology analyser:

Packed cell volume (PCV)	%
Haemoglobin (Hb)	g/dl
Red blood cell count (RBC)	$\times 10^{12}/l$
Absolute indices were calculated as follows:	
Mean corpuscular haemoglobin concentration (MCHC)	
$Hb (g/dl) \times 100 \div PCV (\%)$	g/dl
Mean corpuscular volume (MCV)	
$PCV (\%) \times 10 \div RBC (\times 10^6/mm^3)$	fl
Mean corpuscular haemoglobin (MCH)	
$Hb (g/dl) \times 10 \div RBC (\times 10^{12}/l)$	pg
Total white cell count (WBC total)	$\times 10^9/l$
Differential WBC count (Diff):	
Neutrophils (N)	
Lymphocytes (L)	
Eosinophils (E)	
Basophils (B)	
Monocytes (M)	
Large unstained cells (LUC)	
	$\times 10^9/l$

Units

Cell morphology: the most common morphological changes (anisocytosis, micro/macrocytosis, variation in colour, hypo/hyperchromasia, left shift, atypical/blast cells) were recorded as follows:

- = no abnormalities detected
- + = slight
- ++ = moderate
- +++ = marked

In the case of atypical/blast cells, or other abnormalities, confirmation or a written description from a blood film was made.

Platelet count (Plts) × 10⁹/l

Thrombotest (TT) - Owren, P.A. (Lancet, 1959, *ii*, 754) s

Biochemistry

The following parameters were analysed with a Hitachi 737 Clinical Chemistry Analyser:

Total protein (Protein Total)	g/dl
Albumin (Alb)	g/dl
Globulin (Glob) - by subtraction	
Total protein (g/dl) minus Albumin (g/dl)	g/dl
Urea nitrogen (Urea Nitr)	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic Phosphorus (P)	mEq/l

	Units
Chloride (Cl)	mEq/l
Cholesterol (Chol) - (enzymatic assay)	mg/dl
Alkaline phosphatase (AP) Reaction temperature 30°C	mU/ml
Total bilirubin	mg/dl
Glucose (Hexokinase mediated assay)	mg/dl
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' Reaction temperature 30°C	mU/ml
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' Reaction temperature 30°C	mU/ml
γ Glutamyl transferase (γ GT) Reaction temperature 30°C	mU/ml
Ornithine carbamoyl transferase (OCT) - Method Ceriotti, G., [Clin. Chim. Acta., 1973, 47, 97] Reaction temperature 37°C	mU/ml

TERMINAL STUDIES (Main groups)

On completion of 13 weeks of treatment, all surviving rats were killed.

As the terminal procedures took several days to complete, individual animals continued to receive the appropriate control or test diet until the day on which they were killed. The duration of the dosing period, however, was reported as 13 weeks.

All rats were killed by carbon dioxide asphyxiation and subjected to the necropsy procedure indicated below:

All superficial tissues were examined visually and by palpation and the cranial roof removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral midline incision and skin reflection all subcutaneous tissues were examined. The condition of the thoracic viscera were noted with due attention to the thymus, lymph nodes and heart.

The abdominal viscera were examined before and after removal, the urinary bladder was examined externally and by palpation. The gastrointestinal tract was examined as a whole and the stomach and caecum were incised and examined. The lungs were removed and all pleural surfaces examined under suitable illumination. The liver was sectioned at intervals of a few millimetres; the kidneys were incised and examined. Any abnormalities in the appearance and size of the gonads, adrenals, uterus, intra-abdominal lymph nodes and accessory reproductive organs were recorded.

The following organs from all animals killed at the scheduled sacrifice were dissected free of fat and weighed:

adrenals	liver	spleen
brain	ovaries	testes
epididymides	pituitary	thyroid
heart	prostate	uterus
kidneys	seminal vesicles	

Testes and epididymides were weighed individually and identified as left or right.

The weights of major organs of individual rats dying or killed during the study were recorded at the discretion of the pathologist.

Preservation of tissues

Samples of all the tissues listed below from all animals were preserved. Eyes were preserved in Davidson's fixative. Testes/epididymides were fixed in Bouin's solution and then transferred to 70% alcohol. All other tissues were preserved in buffered 10% formalin.

In addition, samples of any macroscopically abnormal tissues were routinely preserved, along with samples of adjacent tissue where appropriate.

adrenals*	heart*	skin
alimentary tract*	kidneys*	spinal column (to preserve samples of spinal cord from cervical level)
(oesophagus, stomach duodenum, jejunum, ileum, caecum, colon, rectum)	larynx and pharynx	spleen*
aorta*	liver*	sternum* (for bone and marrow)
brain* (medullary, cerebellar and cerebral sections)	lungs* (all lobes and mainstem bronchi)	testes*
epididymides*	lymph nodes* (cervical and mesenteric)	thymus* (where present)
eyes	mammary gland	thyroid* (with parathyroid)
femur (with joint)	ovaries*	tongue
Harderian gland	other macroscopically abnormal tissue*	trachea*
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lacrimal gland and Zymbal's gland)	pancreas*	urinary bladder*
	pituitary*	uterus* (corpus and cervix)
	prostate*	vagina
	salivary gland*	
	sciatic nerve*	
	seminal vesicles*	
	skeletal muscle	

* Tissues required for histopathological examination

Histopathological examination

Tissues required for microscopic examination in this study are marked '*' in the above list. For testes and epididymides, tissues were embedded in paraffin wax and sections were stained with PAS-haematoxylin. A transverse section of each testis and a full longitudinal section of each epididymis was cut as near as possible to 2 micrometres. Microscopic assessment of the testes was made with reference to the stages of the cycle of the seminiferous epithelium. Other tissues were embedded in paraffin wax and sections cut at 4 micrometres were stained with haematoxylin and eosin.

Frozen sections of liver, fixed in buffered formalin, were cut on a cryostat at 12 micrometres and stained for fat with Oil Red O (ORO). Sections of kidney were stained with ORO or Periodic acid-Schiff reagent (PAS) at the discretion of the pathologist.

Histopathological examinations were restricted to:

The specified list of tissues including all macroscopically abnormal tissues from all animals from the control group, and all animals from the high dosage level group, whether dying or killed at 13 weeks.

Any macroscopically abnormal tissue in any animal.

Lungs, liver and kidneys from all rats in the low and intermediate dosage level groups.

TERMINAL STUDIES (Satellite animals)

On completion of 13 weeks of treatment, Satellite group animals were killed.

All animals were perfused and tissue samples were taken from them, but neuropathological examination was restricted to the control and high dose groups.

Method of sacrifice and perfusion fixation: the animals were anaesthetised with sodium pentobarbital (ip) and perfused *in situ* with heparinised 0.7% sodium nitrite followed by a 1.5 glutaraldehyde : 4% paraformaldehyde solution. After perfusion, the cranial vault was removed and the brain removed, weighed and measured as detailed below. The skin was removed from the dorsal region and hindlimbs, and the sciatic, tibial and sural nerves were exposed. The spinal column and cord was fixed *in toto* integral with the carcass overnight in fixative-filled containers and held at 4°C.

Anatomical measurements: the brain was transected from the spinal cord above the first cervical spinal nerve, and the olfactory lobes were removed. The length was measured between the rostral part of the cerebral hemispheres to the most caudal part of the cerebellum, and the width between the widest parts of the cerebral hemispheres.

Following overnight storage, tissues for each animal for paraffin wax H&E sections were taken and stored for at least 48 hours in buffered formalin. Peripheral nerve samples were taken and processed for epon/toluidine blue sections. The carcass was transferred to buffered formalin and stored at room temperature.

Light microscopic examination: brain, spinal cord, ganglia and dorsal and ventral root fibres of the perfused animals were processed for paraffin embedding, sectioned at approximately 5 - 6 microns and stained with haematoxylin and eosin. Peripheral nerves from the right side were embedded in epon, sectioned at approximately 2 microns, and stained with toluidine blue.

Lesions were graded as to severity, where possible, into 4 categories (trace, minimal, moderate or marked).

The tissues for examination are as shown below:

Paraffin wax/H&E sections

Forebrain (three levels) (1))	
Mid-brain (1))	6 sections of brain in total
Cerebellum and pons (1))	
Medulla oblongata (1))	

Spinal cord (cervical (C3-C6) and lumbar (L1-L4) swellings) (2)

Gasserian (trigeminal) ganglia

Dorsal root ganglia (one cervical and one lumbar level))	taken and processed as one
Dorsal root fibres (one cervical and one lumbar level) (3))	sample, from each level
Ventral root fibres (one cervical and one lumbar level) (3))	

The dorsal and ventral root fibres and dorsal root ganglia examined in each case were one from any of C3 - C6 and one from any of L1 - L4.

Epon/Toluidine blue sections

Sciatic nerve (sciatic notch and mid-thigh) (2)

Sural nerve (at and just distal to knee) (2)

Tibial nerve (at and just distal to knee) (2)

- (1) Cross sections of these tissues were evaluated.
- (2) Cross and longitudinal sections of these tissues were evaluated.
- (3) Longitudinal sections of these tissues were evaluated.

STATISTICAL ANALYSIS

All statistical analyses were carried out separately for males and females.

For all parameters the analyses were carried out using the individual animal as the basic experimental unit.

Food and water consumption data were analysed using cumulative cage totals, and bodyweight data were analysed using weight gains.

The following sequence of statistical tests were used for food and water consumption, bodyweight, laboratory investigations and organ weights:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by Fisher (1950). Otherwise:

A test was applied to test for heterogeneity of variance between treatments, Bartlett (1937). Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, an analysis of ranks was used, Kruskal-Wallis (1952/3).

Except for predose data, analyses of variance were followed by Student's *t* test and Williams' test (Williams, 1971/2) for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the *t* test and Williams' test, Shirley's (1977).

Where appropriate for organ weight data, analysis of covariance was used in place of analysis of variance in the above sequence. For this analysis, terminal bodyweight was used as the covariate when the within group relationship between organ weight and bodyweight was significant at the 10% level.

Summary statistics (*eg* means and standard deviations) presented in the report were calculated from computer-stored individual raw data. The summary statistics and the individual data were stored in the computer to a certain number of decimal places, different for each parameter. For presentation purposes, however, they were usually rounded to fewer places. It is therefore, in general, not possible to reproduce the presented means and standard deviations exactly using the presented individual data.

Analysis and presentation of the behavioural screening data

The following data were routinely subjected to statistical analysis: rearing and activity counts, grip strength, hindlimb splay, bodyweight and temperature. These data were analysed using a one-way analysis of variance followed by Williams' test (Williams 1971/2) for a dose-related response. Pre-dose data was analysed by analysis of variance followed by Student's *t* test.

The reporting of the categorical data for the observational battery has been handled in the following manner. The observational endpoints such as ease of handling, arousal, *etc* have been tabulated for frequency of occurrence for each group. Although during recording, some responses were classified in terms of the degree or type of response (*ie* startle: no reaction, an ear twitch, a flinch, *etc*), for the purposes of reporting, as there were no remarkable differences between the groups, for a given endpoint the response has been reported as being either present or absent.

The categorical data were analysed in the following manner:

Where the recorded observations suggested a possible difference between controls and treated groups, the data were analysed using the Linear by Linear Association test (Agresti, 1990; Agresti, Mehta and Patel, 1990). The data for arousal was analysed using Jonckheere-Terpstra test (Hollander & Wolfe, 1973; StatXact, 1992). All the recorded categories were used in the analysis. A two tailed test was generally reported except where the responses were considered directional in nature.

The Coulbourn activity data were analysed using a one-way analysis of variance followed by Williams' test (Williams 1971/2) for a dose-related response. Pre-dose data were analysed by analysis of variance followed by Student's *t* test.

LOCATION OF STUDY RECORDS

All specimens, raw data and other documents generated at Huntingdon during the course of this study, together with a copy of the final report, will be lodged in the Huntingdon Life Sciences Archives, Huntingdon, England.

By 31 December 1997, all raw data and study related documents (but not specimens) will be transferred to EPL Archives Inc, Sterling, Virginia, USA.

PROCEDURES

The procedures used during the study were those documented in the relevant Huntingdon Life Sciences Procedures Manuals.

DEVIATIONS FROM PROTOCOL

There were no deviations from protocol that were considered to have affected the scientific integrity of the study. However, the following minor deviations were noted:

Animals excess to requirements were not discarded until 48 hours after commencement of treatment.

Statistical analysis of water consumption measurements was performed as a routine procedure.

RESULTS

FORMULATION ANALYSIS

The results of the analysis of the diet samples from Weeks 1 and 11 were in good agreement with the intended nominal concentrations (see **NBPT FORMULATION ANALYSIS**) with the results being within 4% of nominal concentration.

CLINICAL SIGNS AND MORTALITIES (Appendix 11)

The only clinical signs considered associated with treatment were limited to one female being treated at 5000 ppm. This animal was observed as having a range of signs including unsteady gait, hunched posture and piloerection. (Similar signs were seen in a palatability study at dosages of 10000 and 20000 ppm, see IMF 6/961820.) All these signs had disappeared by Week 10 of the study.

There was a higher incidence of males treated at 5000 ppm with crooked teeth (2, 1, 2 and 5 in Groups 1 to 4 respectively). However, this was considered to be coincidental and not of toxicological importance.

There was one mortality, during the course of the Week 5 blood sampling procedure, a control female failed to recover from the anaesthetic.

BODYWEIGHTS (Figure 1, Table 1, Appendix 1)

Bodyweight gains among males and females treated at 5000 ppm were lower than controls from the commencement of treatment with the cumulative gains from Week 0 to 4 being statistically significantly lower than controls. During Weeks 4/5, gains for both males and females became generally comparable with controls. The gains for females during Weeks 4 to 8 were statistically significantly increased compared with controls. Towards the end of the study (Weeks 8 to 12), there was a suggestion among males at 5000 ppm of a further slight reduction in weight gains.

At lower dosages, the pattern of weight gains was comparable with controls.

FOOD CONSUMPTION (Table 2, Appendix 2)

Cumulative food intakes were statistically significantly lower among males at 5000 ppm compared with controls. There was no similar effect among females, although it was noted that during the first few weeks of treatment intake was slightly lower when compared with controls.

At lower dosages, intake was comparable with controls.

EFFICIENCY OF FOOD UTILISATION (Table 3)

Treatment at 5000 ppm was initially associated with an impairment of food utilisation. Among males, there was evidence of increasing impairment from the commencement of treatment through to Week 3. Among females, there was a very marked impairment during Weeks 2 and 3. From Week 4 onwards, food utilisation showed a complete recovery with values being comparable to or better than controls.

At lower dosages, values were considered comparable with controls.

ACHIEVED MEAN INTAKE (Table 4)

The achieved mean intake of test material was as expected in a fixed inclusion level study. There was no overlap between the selected dosages and generally there was a five-fold interval in the achieved intakes.

The overall achieved intake in terms of mg/kg/day was 14.7, 74, 377 for Main group males and 17.4, 88 and 445 for Main group females.

WATER CONSUMPTION (Table 5, Appendix 3)

There were no statistically significant differences in water consumption between treated and control groups.

OPHTHALMOSCOPY (Appendix 6)

The ophthalmoscopic findings were within normal limits for animals of this age and strain. None of the changes observed were considered related to treatment.

NEUROBEHAVIOURAL OBSERVATIONS**Observational endpoints (Table 6, Appendix 4)**

Emaciated appearance was recorded on all occasions with statistically significant differences in the incidence of observations occurring during Weeks 4 and 8 for both sexes treated at 5000 ppm and for females only during Week 13.

During Week 4, hunched posture was observed in two females at 5000 ppm. The observation of grooming while in the arena was statistically significantly higher among males treated at 5000 ppm compared with controls, however, no toxicological importance is attached to this singular observation.

Among males treated at 1000 or 5000 ppm, there was a statistically significant increase in the numbers of animals vocalising on removal from the cage compared with controls during Week 8. During Week 13, although there was no dosage relationship, all treated males were more likely to vocalise compared with controls.

Among males treated at 5000 ppm, there was a slight shift to a lower level of arousal compared with controls. This was first observed in Week 8. It was still present in Week 13 with differences from control just failing to attain statistical significance.

There was an increase in the incidence of males at 5000 ppm for which assessment of gait was not possible due to limited movement in the arena. This was observed in Week 8 and 13 with the incidence attaining statistical significance in Week 13.

During Week 13, there was a statistically significant increase in the incidence of slight eye closure in the arena among males treated at 5000 ppm.

Singular observations made during the course of the study but which could not be clearly attributed to treatment included the following:

cold feet: Week 4 one male at 200 ppm and one male at 1000 ppm; Week 8 one female at 200 ppm;

firm abdomen: Week 4 one male at 5000 ppm, Week 8 one male at 1000 ppm

slightly swollen abdomen: Week 8 one female at 5000 ppm

rapid breathing: Week 4 one male at 5000 ppm.

Rearing and activity counts (Tables 7 and 8, Appendix 4)

There were no statistically significant differences in rearing or activity counts between treated and control groups throughout the treatment period.

Grip strength (Tables 9 and 10, Appendix 4)

During Week 4, there were statistically significant lower values recorded for both forelimb and hindlimb grip strength among males and females treated at 5000 ppm. During Weeks 8 and 13 no statistically significant differences in grip strength, although it was noted in Week 8 that females treated at 5000 ppm did show slightly lower mean grip strength values when compared with controls.

At lower dosages, values were comparable with controls throughout treatment.

Hind limb splay (Table 11, Appendix 4)

There were no statistically significant differences in mean splay values between treated and control groups throughout the treatment period.

Rectal temperature (Table 12, Appendix 4)

There were no statistically significant differences in mean rectal temperature between treated and controls throughout the treatment period.

Bodyweight (Table 13, Appendix 4)

During Week 4, statistically significant lower mean bodyweights were recorded for males and females treated at 5000 ppm compared with their respective controls. At lower dosages, bodyweights were comparable with controls. During Week 8 and 13, statistically significant lower mean bodyweights were recorded for males treated at 5000 ppm compared with controls. During Week 8 and 13, among females at 5000 ppm, bodyweights remained lower than controls but differences failed to attain statistical significance.

At lower dosages, there were no statistically significant differences from controls.

Coulbourn activity monitoring (Table 14, Appendix 5)

There were no statistically significant differences in activity among treated and controls animals throughout the treatment period.

Discussion

Treatment with NBPT for thirteen weeks was associated with some behavioural changes as well as clear evidence of toxicity as evidenced by effects on bodyweight. The behavioural changes and evidence of toxicity were mainly confined to treatment at the highest dosage, 5000 ppm. The observations appeared to change during the course of exposure.

During Week 4, there were clear effects on bodyweight (lower weights) and an observation in some animals at 5000 ppm of emaciated appearance. There was a statistically significant lower grip strength values for both males and females at 5000 ppm. This may partly reflect the effect on bodyweight, however, the degree of the effect on hindlimb grip strength among females at 5000 ppm could not solely be related to bodyweight as the bodyweight at Week 4 was greater than in the predose period and yet the grip strength in Week 4 was lower than the predose period. At later weeks, although bodyweights continued to be lower than controls, grip strength values recovered to control levels and there were no statistically significant differences from controls. Other changes in Week 4 included a low incidence of hunched posture among females at 5000 ppm.

During Week 8 and 13, there was a tendency for more treated males to vocalise when being handled compared with controls. This was interpreted as possibly a non-specific effect of treatment.

During Week 13, among males treated at 5000 ppm, there were observations of slight eye closure in the arena, an increase in the number of animals for which assessment of gait was not possible and a tendency for a lower level of arousal. These signs of slight eye closure and lack of gait assessment were considered to be related to the lower arousal evidence these animals. With lower arousal, the expectation would be for lower activity and rearing counts, however, there were no statistically significant effects on either behaviour nor was there any significant effect in activity in the Coulbourn activity system. In the absence of changes in other behaviours, the changes in arousal and associated effects are therefore considered to be of doubtful toxicological significance.

To conclude, treatment with NBPT for thirteen weeks was associated with a transitory change in behaviour during Week 4 which was characterised by decreased grip strength for all animals treated at 5000 ppm and a low incidence of hunched posture in females at 5000 ppm. With continuing exposure, these changes disappeared.

HAEMATOLOGY (Table 15, Appendix 7)

Week 5

Among males at 5000 ppm, the total white blood cell counts were statistically lower than controls. This was attributed to the statistically significantly lower lymphocyte counts at 5000 ppm. Among females, there was a statistically significant increase in platelet counts at 5000 ppm.

Week 13

Among females at 5000 ppm, there was a statistically significant increase in platelet counts at 5000 ppm.

Other statistically significant differences from control values were observed in Weeks 5 and 13 but were considered to be marginal in nature and of no toxicological importance.

BIOCHEMISTRY (Table 16, Appendix 8)

Week 5

Among males, GPT values were statistically significantly decreased at 5000 ppm, and GOT values were statistically significantly decreased at 1000 and 5000 ppm. Phosphorus levels were also statistically significantly lower at 5000 ppm.

Among females, AP, GPT and GOT values were statistically significantly decreased at 5000 ppm compared with controls.

Week 13

Among males, AP values were statistically significantly decreased at 1000 and 5000 ppm. GOT values were statistically significantly decreased at 5000 ppm. Phosphorus levels were also marginally, but statistically significantly lower at 5000 ppm.

Among females, GPT values were statistically significantly decreased at 5000 ppm compared with controls. (Although mean GPT values at 1000 ppm, were also lower than controls, the values were highly variable and failed to attain statistical significance.)

Among females phosphorus levels were statistically significantly decreased at all dosages of NBPT. It was noted that there was a wide range of values for the controls. Examination of background data showed a 95% range of 2.4 - 4.3 with a mean of 3.3. Values for females treated at 200 and 1000 ppm were therefore within background.

Other statistically significant differences from control values were observed in Weeks 5 and 13 but were considered to be marginal in nature and of doubtful toxicological significance.

ORGAN WEIGHTS (Table 17, Appendix 9)

Among males at 5000 ppm, there was a statistically significant increase in liver weights when adjusted for bodyweights. Among females, there was a statistically significant increase in uterus weights compared with controls at 1000 and 5000 ppm.

MACROSCOPIC PATHOLOGY (Table 19, Appendix 11)

The only change noted at macroscopic examination at terminal autopsy was uterine fluid distension in 0, 0, 5, 6 females in Groups 1 to 4 respectively. There were no other macroscopic changes which were considered related to treatment.

BRAIN MEASUREMENTS (Table 18, Appendix 10)

There were no statistically significant differences from controls in any of the brain measurements.

MICROSCOPIC PATHOLOGY**Terminal kill - Main group animals - toxicity screen**

Treatment-related changes

Liver - Minimal centrilobular hepatocyte hypertrophy was reported in male rats at 1000 and 5000 ppm.

	Male			
Dosage level (ppm)	0	200	1000	5000
Minimal centrilobular hepatocyte hypertrophy	0	0	2	6**
Number livers examined	10	10	10	10

** $p < 0.01$ with Fisher's Exact Test

This finding attained statistical significance in male rats at 5000 ppm and was considered to be treatment-related in this group. The incidence reported in male rats at 1000 ppm was within the limits of background histopathological findings reported in this age and strain of laboratory-maintained rats.

Minimal centrilobular hepatocyte hypertrophy was reported in a proportion of control and treated female rats but was not considered to be treatment-related.

Uterus - Luminal dilatation was reported in all treated groups of female rats.

	Female			
Dosage level (ppm)	0	200	1000	5000
Luminal dilatation	0	3	5	6
Number uteri examined	9	3	5	10

This finding showed a dose-related incidence and was considered to be treatment-related in all treated groups of rats.

Although fluid distension of the uterus is a normal physiological event associated with oestrus cycling, the significance of its occurrence in treated groups is unknown.

Kidneys - Foci of dystrophic mineralisation were reported in the kidneys in a proportion of control and treated female rats.

Dosage level (ppm)	Female			
	0	200	1000	5000
Dystrophic mineralisation				
Total	2	2	2	6
Minimal	2	2	1	3
Moderate	0	0	1	3
Number kidneys examined	9	10	10	10

Although this finding did not attain statistical significance in any group there was an increased incidence and severity of mineralisation in the 5000 ppm group which may be related to treatment. Foci of mineralisation in the kidneys of female rats is a sex-related spontaneous finding considered to be related to circulating oestrogen levels. In view of the uterine findings in treated females, it is possible that the increase in renal mineralisation at 5000 ppm may reflect an altered hormonal balance.

Terminal kill - Satellite animals -neurotoxicity screen

No treatment-related effects were reported in any tissues available for histopathological examination.

Conclusion

Terminal kill main group animals - Treatment-related findings were reported in the liver, uterus and kidneys.

Liver - Centrilobular hepatocyte hypertrophy in male rats receiving 5000 ppm.

Uterus - Luminal dilatation in all treated groups of rats.

Kidneys - Foci of mineralisation in female rats receiving 5000 ppm.

Terminal kill satellite animals - No treatment-related findings were reported.

DISCUSSION AND CONCLUSION

Treatment with NBPT, a urease inhibitor, for thirteen weeks at dietary inclusion levels of 0, 200, 1000 and 5000 ppm was associated with a range of effects principally at 5000 ppm.

For some of the observations at 5000 ppm, the pattern of reaction to treatment changed with continuing exposure such that an initial reaction was followed by recovery. This included a single female which showed clinical signs up to Week 10 of the study; an initial reduction in bodyweight gains, an impairment in food utilisation for the first 4 weeks of treatment, and observations on the functional observational battery when performed in Week 4 but not at subsequent intervals. Other observations showed a continuing effect such as the lower cumulative food consumption among males at 5000 ppm.

Treatment at 5000 ppm was also associated with effects on some haematology, and biochemistry parameters. Organ weight changes were limited to increased liver weights for males and increased uterine weights for females. The increased liver weights was associated with centrilobular hepatocyte hypertrophy and the increased uterine weight was associated with luminal dilatation of the uterus. There was also an increase in the incidence of foci of mineralisation in the kidney among females.

The behavioural assessment showed transitory changes in Week 4 at 5000 ppm but there were no subsequent changes. Neuropathology after 13 weeks of exposure provided no evidence of neurotoxicity of NBPT.

At 1000 ppm, effects were limited to changes in some biochemistry parameters in both males and females and effects on the uterus (luminal dilatation) among females.

At 200 ppm, effects were limited to females and included statistically significantly lower phosphorus levels (which was within background) and a low incidence of luminal dilatation of the uterus.

To conclude, treatment with NBPT for thirteen weeks was associated with clear signs of toxicity at 5000 ppm. At 1000 ppm, there were limited effects on some biochemistry parameters and among females, an increased incidence of luminal dilatation of the uterus.

The no observed effect level (NOEL) was established in the males as being 200 ppm. It was not possible to demonstrate a NOEL among females as a slightly lower phosphorus level and a low incidence of luminal dilatation of the uterus was observed. The toxicological significance of these findings, if any, cannot be determined at this time.

REFERENCES

- AGRESTI, A. (1990) Categorical data analysis. John Wiley & Sons, New York.
- AGRESTI, A., MEHTA, C.R., PATEL, N.R. (1990) Exact interference for contingency tables with ordered categories. *J. Amer. Stat.Ass.* **85**: 453 - 458.
- BARTLETT, M.S. (1937) Properties of sufficiency and statistical test. *Proc. Roy. Soc., A* **160**, 268.
- CERIOTTI, G. (1973) Optimal conditions for ornithine carbamyl transferase determination. A simple micromethod without deproteinization. *Clin. Chem. Acta.*, **47**, 97.
- FISHER, R.A. (1950) in: (Eds). *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh.
- HOLLANDER, M. and WOLFE, D.A. (1973) *Non-parametric statistical methods*. John Wiley & Sons, New York.
- KRUSKAL, W.H. and WALLIS, W.A. (1952) Use of ranks in one-criterion variance analysis. *J. Amer. Statist. Ass.*, **47**, 583.
- KRUSKAL, W.H. and WALLIS, W.A. (1953) Errata, *J. Amer. Statist. Ass.*, **48**, 907.
- OWREN, P.A. (1959) A new method for controlling antiocagulant therapy. *Lancet*, **ii**, 754.
- SHIRLEY, E. (1977) A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics*, **33**, 386.
- StatXact-Turbo User Manual (1992), Cytel Software Corporation, Cambridge MA.
- WILLIAMS, D.A. (1971/2), *Biometrics*, **27**: 103 - 117 and **28**: 519 - 531.

FIGURE 1
Bodyweights - group mean values

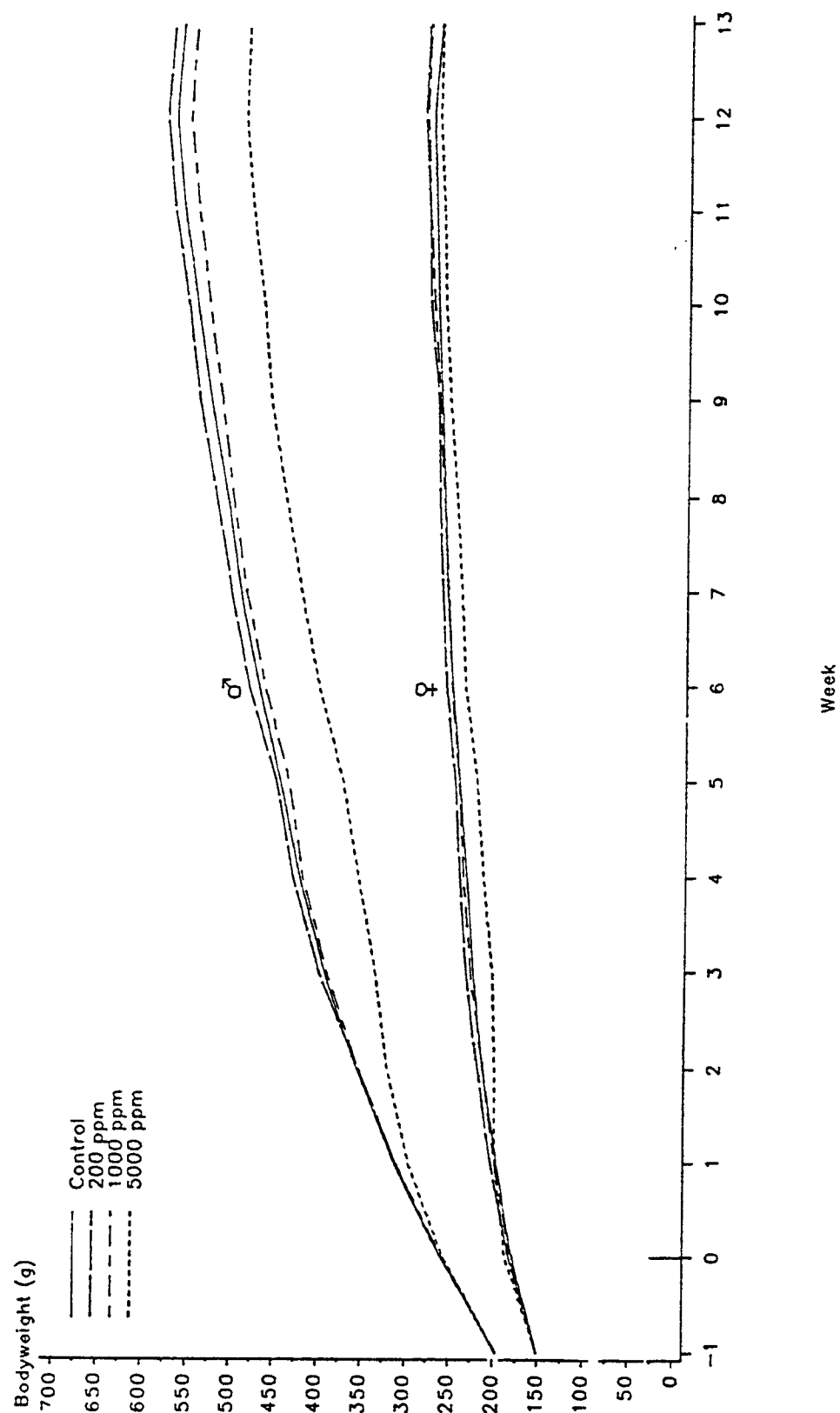


TABLE 1

Bodyweights - group mean values (g/rat/week) - Main group

Week	Group/ dosage (ppm) NBPT							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
-1	196	197	196	197	151	151	152	151
0	259	259	258	256	180	183	181	187
1	314	312	311	297	198	205	200	200
2	356	354	354	322	215	220	214	201
3	395	401	391	337	226	235	229	205
4	424	431	420	357	235	243	238	216
5	446	450	436	375	243	249	244	224
6	472	483	464	403	253	259	253	237
7	494	504	486	424	259	264	258	243
8	509	523	504	443	263	269	264	249
9	531	543	518	461	268	272	270	258
10	547	557	533	470	273	282	277	264
11	563	574	546	484	276	284	283	267
12	573	584	557	493	280	290	287	273
13	566	576	551	490	271	285	283	271
Cumulative gains (g) Week								
0 to 4	165	172	162	100**	55	59	57	29**
4 to 8	85	91	84	86	25	26	26	34*
8 to 12	64	61	54	51	17	21	23	24
0 to 12	314	324	300	237**	99	106	107	86
sd								
0 to 4	14.0	24.1	16.5	19.4	12.6	9.1	21.0	11.6
4 to 8	16.1	16.0	11.0	23.0	6.6	7.7	8.0	11.0
8 to 12	14.4	14.9	16.8	12.8	7.4	8.1	7.7	10.2
0 to 12	32.4	47.9	30.1	34.8	17.4	20.9	31.9	19.5
% of control								
0 to 4		104	98	61		107	104	53
4 to 8		107	99	101		104	104	136
8 to 12		95	84	80		124	135	141
0 to 12		103	96	75		107	108	87

sd Standard deviation

* $p < 0.05$, ** $p < 0.01$

(Note: Week 13 not analysed due to laboratory investigations)

TABLE 1

(Bodyweights - continued - Satellite group)

Week	Group/ dosage (ppm) NBPT							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
-1	191	192	192	192	157	157	156	157
0	252	251	253	253	185	183	184	189
1	305	302	306	290	211	207	202	206
2	346	337	342	329	229	230	220	212
3	379	378	378	351	246	241	233	214
4	400	402	404	373	260	248	243	223
5	426	428	430	396	270	261	253	236
6	451	456	458	420	281	275	262	247
7	470	477	480	441	282	278	270	251
8	484	493	496	447	291	283	272	259
9	497	514	513	440	299	291	277	265
10	512	533	528	455	308	299	285	271
11	524	544	545	469	311	301	289	274
12	530	555	559	478	314	306	291	276
13	534	561	560	483	315	308	291	277
Cumulative gains (g)								
Week								
0 to 4	147	151	152	120	75	65	58	34**
4 to 8	85	92	92	74	30	35	29	36
8 to 12	46	62	62	31	24	22	19	17
0 to 12	278	304	306	225	129	123	107	87
sd								
0 to 4	24.3	27.0	14.6	21.7	8.9	16.7	24.8	12.2
4 to 8	12.3	22.0	15.8	23.4	5.8	12.7	9.1	11.6
8 to 12	6.9	12.0	18.9	20.3	8.6	7.4	7.9	11.1
0 to 12	19.9	51.1	29.4	43.6	22.7	34.5	39.6	29.7
% of control								
0 to 4		103	103	82		87	77	45
4 to 8		108	108	87		117	97	120
8 to 12		135	135	67		92	79	71
0 to 12		109	110	81		95	83	67

sd Standard deviation

** $p < 0.01$

(Note: Week 13 not analysed to match Main group)

TABLE 2

Food consumption - group mean values (g/rat/week) - Main group

Week	Group/ dosage (ppm) NBPT							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
-1	206	201	200	199	145	146	148	152
1	236	229	227	210	155	161	160	147
2	230	234	232	214	154	162	159	147
3	238	247	241	209	158	165	165	147
4	235	249	244	211	160	169	165	155
5	215	221	214	193	142	145	143	140
6	237	244	232	217	148	151	150	150
7	226	232	227	206	147	151	149	147
8	225	236	231	210	148	150	150	148
9	230	237	224	211	140	141	145	144
10	227	232	218	199	141	150	147	140
11	227	229	221	202	139	142	147	140
12	224	228	216	199	136	148	146	146
13	201	206	203	190	124	134	131	129
Cumulative intake (g) Week 1 to 12	2753	2822	2724	2486**	1779	1833	1824	1750
sd 1 to 12	164.8	233.9	161.2	221.5	111.4	151.8	200.3	146.6
% of controls 1 to 12		103	99	90		103	103	98

sd Standard deviation

** $p < 0.01$

(Note: Week 13 not analysed due to laboratory investigations)

TABLE 2

(Food consumption - continued - Satellite group)

Week	Group/ dosage (ppm) NBPT							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
-1	194	197	197	186	146	143	152	150
1	227	232	224	194	155	162	155	150
2	222	231	219	205	160	162	161	153
3	221	240	225	211	169	166	170	151
4	218	240	223	208	172	171	169	155
5	223	235	223	209	160	164	163	146
6	215	229	220	204	162	161	163	147
7	213	222	218	204	152	151	156	139
8	215	219	214	190	149	147	147	138
9	216	231	218	173	154	154	146	141
10	209	227	209	166	149	150	151	133
11	216	223	215	183	151	147	148	134
12	211	220	219	182	149	150	145	131
13	203	214	209	190	139	151	141	131
Cumulative intake (g) Week 1 to 12	2649	2771	2647	2347	1926	1889	1895	1744
sd 1 to 12	92.1	301.2	244.2	184.8	79.2	211.9	232.1	182.2
% of controls 1 to 12		105	100	89		98	98	91

sd Standard deviation

No statistical significance ($p > 0.05$)

(Note: Week 13 not analysed to match Main group)

TABLE 3

Food conversion ratios - group mean values - Main group

Week	Group and diet concentration (ppm)							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
1	4.3	4.4	4.3	5.1	8.6	7.4	8.3	11.3
2	5.4	5.6	5.4	8.7	9.0	10.6	11.7	179.1
3	6.2	5.2	6.5	14.0	13.4	11.3	10.6	39.3
4	8.0	8.2	8.6	10.6	19.9	21.6	18.7	14.1
5	9.8	11.6	13.1	10.8	28.8	24.8	25.6	17.8
6	9.2	7.5	8.3	7.6	15.3	14.1	16.2	10.9
7	10.4	10.9	10.1	9.9	23.3	29.0	29.9	27.4
8	14.1	12.8	13.4	11.3	38.4	32.6	25.9	22.5
9	10.8	11.4	15.7	11.4	28.9	49.9	23.2	16.3
10	14.4	16.9	14.1	23.1	27.3	15.2	20.1	22.1
11	13.7	13.9	16.9	14.5	38.2	62.0	24.6	50.2
12	22.0	22.3	19.5	21.0	38.5	26.5	37.8	25.3
13	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1 - 4	6.0	6.2	5.9	6.8	8.8	10.1	11.2	18.0
5 - 8	10.3	9.9	9.5	10.9	20.6	17.8	21.6	15.7
9 - 12	18.4	14.6	13.8	22.5	25.5	26.9	30.8	31.0

n/a Insufficient bodyweight gain to give a meaningful ratio

Food conversion ratio = food consumption/bwt gain

(Note: Week 13 laboratory investigations performed)

TABLE 3

(Food conversion ratios - continued - Satellite group)

Week	Group and diet concentration (ppm)							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
1	4.3	4.5	4.2	5.1	6.0	6.7	8.7	9.0
2	5.5	6.6	6.0	5.3	8.8	7.2	9.2	22.5
3	6.7	5.9	6.3	9.6	10.3	14.1	12.9	85.7
4	10.6	10.1	8.5	9.4	11.9	24.9	17.3	18.4
5	8.3	8.9	8.6	9.1	16.0	12.7	16.1	11.2
6	8.7	8.0	7.8	8.3	15.3	11.2	17.6	12.6
7	11.0	10.5	10.0	9.8	149.3	66.7	18.4	33.3
8	15.7	14.1	13.3	34.9	17.2	26.6	116.6	18.7
9	16.3	11.0	13.1	n/a	17.7	19.9	27.7	21.8
10	14.7	12.2	13.9	11.4	16.3	18.0	18.3	22.9
11	17.4	19.6	12.6	12.7	58.1	88.8	34.4	44.9
12	33.6	20.8	15.9	20.5	46.7	32.4	111.4	61.8
13	63.0	34.8	248.5	39.5	173.6	64.9	261.1	298.3
1 - 4	5.7	5.6	5.8	8.4	11.4	11.1	11.3	20.9
5 - 8	10.6	10.2	10.8	9.6	23.6	22.6	23.1	17.4
9 - 12	14.2	15.1	16.3	16.0	32.4	28.3	25.0	24.0

n/a Insufficient bodyweight gain to give a meaningful ratio

Food conversion ratio = food consumption/bwt gain

(Note: Week 13 laboratory investigations performed on the Main group)

TABLE 4

Achieved group mean intakes of test substance (mg/kg/day) - Main group

Week	Group and diet concentration (ppm)					
	2♂ 200	3♂ 1000	4♂ 5000	2♀ 200	3♀ 1000	4♀ 5000
1	22.9	114	541	23.7	120	543
2	20.1	100	493	21.7	110	523
3	18.7	92	453	20.8	107	518
4	17.1	86	434	20.3	101	526
5	14.3	72	378	16.9	85	454
6	15.0	74	399	17.0	86	465
7	13.4	68	356	16.5	83	438
8	13.2	67	346	16.0	82	429
9	12.7	63	333	14.9	77	405
10	12.0	59	305	15.4	77	383
11	11.5	58	303	14.3	75	376
12	11.2	56	291	14.7	73	386
13	10.1	52	276	13.3	66	339
1 to 13	14.7	74	377	17.4	88	445

TABLE 4

(Achieved group mean intakes of test substance - continued - Satellite group)

Week	Group and diet concentration (ppm)					
	2♂ 200	3♂ 1000	4♂ 5000	2♀ 200	3♀ 1000	4♀ 5000
1	23.9	115	510	23.8	115	544
2	20.6	97	472	21.2	109	523
3	19.2	89	444	20.1	107	505
4	17.6	82	412	19.9	102	507
5	16.2	76	388	18.4	94	454
6	14.8	71	357	17.2	90	434
7	13.6	66	338	15.6	84	398
8	12.9	63	305	14.9	77	385
9	13.1	62	279	15.3	76	385
10	12.4	57	265	14.5	77	353
11	11.8	57	283	14.0	74	351
12	11.4	57	274	14.1	71	340
13	11.0	53	282	14.0	69	339
1 to 13	15.3	73	355	17.2	88	424

TABLE 5

Water consumption - group mean values (g/rat/day) - Main group

Week 12	Group and diet concentration (ppm)							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
Day								
1	36.6	36.4	35.4	36.4	25.0	29.9	29.7	29.3
2	41.5	40.3	34.7	43.3	28.4	29.6	30.4	32.6
3	39.6	41.3	37.9	39.9	28.8	32.7	32.4	28.3
4	41.5	40.0	38.5	41.7	29.3	30.3	30.5	30.7
5	38.5	40.4	35.9	39.3	27.3	28.7	29.5	30.0
6	38.4	39.8	38.1	40.4	26.6	31.8	29.7	30.1
7	38.5	39.2	38.3	41.8	26.7	28.4	32.4	27.3
Total consumption (g/rat/week)								
Week 12	275	277	259	283	192	211	215	208
sd	31.5	64.9	34.2	53.2	39.3	58.8	43.4	55.2
% of control	-	101	94	103	-	110	112	108

sd Standard deviation

TABLE 5

(Water consumption - continued - Satellite group)

Week 12	Group and diet concentration (ppm)							
	1♂ Control	2♂ 200	3♂ 1000	4♂ 5000	1♀ Control	2♀ 200	3♀ 1000	4♀ 5000
Day								
1	40.2	36.8	35.4	41.6	31.6	30.0	28.8	22.8
2	46.8	37.8	37.2	40.4	32.6	28.0	31.0	25.4
3	42.4	37.3	37.8	37.1	31.8	28.6	32.8	25.2
4	42.0	36.4	38.0	39.8	31.6	32.0	32.6	26.8
5	45.2	38.2	37.8	38.2	31.2	31.0	32.8	27.2
6	42.0	39.2	37.0	38.8	30.6	28.8	30.6	24.8
7	40.8	38.6	36.6	39.6	31.2	27.4	33.2	27.8
Total consumption (g/rat/week)								
Week 12	299	264	260	276	221	206	222	180
sd	52.9	26.3	53.1	31.1	45.6	23.4	44.3	28.9
% of control	-	88	87	92	-	93	100	81

sd Standard deviation

TABLE 6

Summary of functional observational battery - Pre-dose

Group	Males				Females			
	1	2	3	4	1	2	3	4
No. of animals	10	10	10	10	10	10	10	10
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	10	10	10	10
REMOVAL FROM CAGE								
removing, easy	10	10	10	10	10	10	10	10
handling, easy	7	10	9	10	10	10	8	9
salivation	1	1	2	1	0	0	1	0
vocalising	1	0	1	2	1	2	2	2
IN THE ARENA								
tremors	0	1	0	1	0	0	0	0
grooming	6	6	3	6	3	4	1	4
arousal, alert	9	8	10	8	9	9	8	9
defecation	1	5	2	2	0	0	0	0
urine	7	4	0	5	2	1	3	1
GAIT								
walking on toes	0	0	0	0	0	0	0	1
swaying	0	0	0	1	0	0	0	0
unable to assess	0	3	0	2	4	1	2	1
MANIPULATIONS								
approach, a reaction	10	10	10	10	10	10	10	10
touch, a reaction	8	10	6	9	8	8	10	10
startle (present)	10	10	10	10	10	10	10	10
righting, immediately	10	9	10	10	9	10	10	9
tail pinch, a reaction	10	10	10	10	10	10	10	10
pupil reflex	10	10	10	10	10	10	10	10

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response or with the indicated score

TABLE 6

Summary of functional observational battery - Week 4

Group	Males				Females			
	1	2	3	4	1	2	3	4
No. of animals	10	10	10	10	10	10	10	10
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	10	10	10	10
REMOVAL FROM CAGE								
removing, easy	10	10	10	10	10	10	9	10
handling, easy	9	8	10	10	10	9	10	10
salivation	2	1	1	0	0	0	1	0
vocalising	0	5	3	3	2	2	3	1
IN THE ARENA								
tremors	3	1	2	2	0	0	0	0
grooming	1	2	3	5a	4	2	2	3
arousal, alert	8	5	9	6	10	10	10	10
defecation	0	2	0	2	0	0	0	0
urine	4	1	2	5	0	1	2	0
GAIT								
walking on toes	0	1	1	2	3	1	4	5
hunched	0	0	0	0	0	0	0	2
unable to assess	2	5	2	2	2	0	0	0
MANIPULATIONS								
approach, a reaction	10	10	10	10	10	10	10	10
touch, a reaction	6	6	7	7	10	8	8	9
startle (present)	10	10	10	10	10	10	10	9
righting, immediately	10	8	10	10	10	10	10	9
tail pinch, a reaction	10	10	10	10	10	10	10	10
pupil reflex	10	9	10	10	10	10	10	10
ADDITIONAL COMMENTS								
emaciated appearance	1	1	0	5b	0	2	2	7c

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response
or with the indicated score

a , p = 0.0475

b, p = 0.0056

c, p = 0.0083

TABLE 6

Summary of functional observational battery - Week 8

Group	Males				Females			
	1	2	3	4	1	2	3	4
No. of animals	10	10	10	10	9	10	10	10
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	9	10	10	10
REMOVAL FROM CAGE								
removing, easy	10	9	10	10	9	10	10	10
handling, easy	9	9	9	8	9	10	9	9
salivation	1	2	0	0	0	0	0	0
vocalising	1	2	5b	6a	1	2	4	2
IN THE ARENA								
tremors	0	1	5	3	0	0	1	1
grooming	1	4	2	2	6	1	3	7
arousal, alert	8	7	6	5	9	10	8	9
defecation	1	0	0	2	0	0	0	0
urine	6	4	4	3	1	0	1	1
GAIT								
walking on toes	0	1	1	1	4	3	3	4
hunched	0	0	0	0	0	0	0	1
swaying	0	0	2	0	0	0	0	1
unable to assess	2	2	4	5	0	0	2	0
MANIPULATIONS								
approach, a reaction	10	9	9	8	9	10	10	10
touch, a reaction	4	9	6	8	8	9	9	10
startle (present)	10	10	10	10	9	10	10	10
righting, immediately	10	8	9	10	8	10	10	9
tail pinch, a reaction	10	10	10	10	9	10	10	10
pupil reflex	10	9	10	10	9	9	9	9
ADDITIONAL COMMENTS								
emaciated appearance	0	0	0	3c	0	1	2	3d

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response
or with the indicated scorea, $p = 0.0134$ b, $p = 0.0318$ c, $p = 0.0121$ d, $p = 0.0436$

TABLE 6

Summary of functional observational battery - Week 13

Group	Males				Females			
	1	2	3	4	1	2	3	4
No. of animals	10	10	10	10	9	10	10	10
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	9	10	10	10
REMOVAL FROM CAGE								
removing, easy	10	10	9	10	9	10	10	10
handling, easy	10	9	8	9	8	10	7	9
salivation	0	0	1	0	0	0	0	0
vocalising	2	7	6	5	3	0	3	2
IN THE ARENA								
tremors	3	3	4	2	1	0	1	1
grooming	1	3	1	2	3	4	5	4
arousal, alert	8	8	5	4d	6	9	8	8
defecation	0	0	0	2	0	0	0	0
urine	5	4	3	4	1	1	1	0
GAIT								
walking on toes	1	0	1	1	2	3	3	2
swaying/lurching	1	1	1	1	0	0	0	0
unable to assess	1	3	2	6a	2	0	2	2
MANIPULATIONS								
approach, a reaction	10	9	9	9	9	10	10	10
touch, a reaction	4	5	4	7	7	8	9	9
startle (present)	10	10	10	10	9	10	10	10
righting, immediately	10	8	10	10	8	10	10	10
tail pinch, a reaction	10	10	10	10	9	10	10	10
pupil reflex	9	10	10	10	9	9	10	9
ADDITIONAL COMMENTS								
emaciated appearance	0	1	0	1	0	1	0	3c
slight eye closure in the arena	1	1	0	4b	0	0	2	0

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response

a, p = 0.0147

d, p = 0.0568

b, p = 0.0234

c, p = 0.0286

TABLE 7

Activity counts - group mean values

Pre-dose

Group/ dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1 (0)	11	5.3	6	3.8
2 (200)	6+	5.4	9	4.9
3 (1000)	9	2.2	9	4.9
4 (5000)	7	3.5	9	3.0

Week 4

Group/ dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1 (0)	6	3.6	13	8.0
2 (200)	7	6.7	14	5.2
3 (1000)	8	3.2	12	6.2
4 (5000)	6	4.5	12	3.9

Week 8

Group/ dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1 (0)	7	5.0	14	7.7
2 (200)	9	6.4	14	7.8
3 (1000)	7	4.4	11	9.0
4 (5000)	5	5.9	12	6.4

Week 13

Group/ dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1 (0)	6	3.0	9	5.9
2 (200)	6	4.2	11	5.5
3 (1000)	6	2.9	11	7.5
4 (5000)	4	3.4	9	5.5

sd Standard deviation

Student's *t* test, + *p* < 0.05

TABLE 8

Rearing counts - group mean values

Pre-dose

Group/ dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1 (0)	10	3.3	8	3.8
2 (200)	7+	3.8	9	3.8
3 (1000)	8	1.7	8	4.3
4 (5000)	8	4.0	9	4.3

Week 4

Group/ dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1 (0)	9	5.1	11	5.7
2 (200)	7	5.6	12	5.0
3 (1000)	7	2.4	12	5.6
4 (5000)	7	4.1	13	2.6

Week 8

Group/ dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1 (0)	7	4.2	13	7.2
2 (200)	8	5.2	12	6.0
3 (1000)	7	4.5	10	6.6
4 (5000)	5	4.0	11	6.6

Week 13

Group/ dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1 (0)	5	3.5	9	4.9
2 (200)	4	4.0	8	3.7
3 (1000)	4	2.2	9	5.3
4 (5000)	4	3.3	7	4.2

sd Standard deviation

Student's *t* test, + $p < 0.05$

TABLE 9

Grip strength - forelimb - group mean values

Pre-dose

Group/ dosage (ppm)	Mean forelimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	0.74	0.113	0.71	0.109
2 (200)	0.76	0.082	0.73	0.132
3 (1000)	0.75	0.088	0.76	0.120
4 (5000)	0.78	0.111	0.72	0.067

Week 4

Group/ dosage (ppm)	Mean forelimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.32	0.287	1.02	0.225
2 (200)	1.32	0.180	1.09	0.209
3 (1000)	1.22	0.100	1.12	0.210
4 (5000)	1.10*	0.264	0.80*	0.173

Week 8

Group/ dosage (ppm)	Mean forelimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.41	0.273	1.11	0.216
2 (200)	1.56	0.136	1.05	0.269
3 (1000)	1.37	0.188	1.20	0.202
4 (5000)	1.59	0.199	1.06	0.319

Week 13

Group/ dosage (ppm)	Mean forelimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.35	0.415	1.21	0.224
2 (200)	1.49	0.238	1.12	0.360
3 (1000)	1.45	0.305	1.21	0.391
4 (5000)	1.54	0.311	1.14	0.235

sd Standard deviation

* $p < 0.05$

TABLE 10

Grip strength - hindlimb - group mean values

Pre-dose

Group/ dosage (ppm)	Mean hindlimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	0.73	0.105	0.75	0.112
2 (200)	0.74	0.089	0.80	0.100
3 (1000)	0.72	0.157	0.79	0.150
4 (5000)	0.79	0.093	0.72	0.150

Week 4

Group/ dosage (ppm)	Mean hindlimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.18	0.118	1.13	0.214
2 (200)	1.21	0.143	1.13	0.233
3 (1000)	1.16	0.208	1.02	0.283
4 (5000)	0.94**	0.263	0.57**	0.163

Week 8

Group/ dosage (ppm)	Mean hindlimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.21	0.217	1.06	0.359
2 (200)	1.31	0.183	1.10	0.260
3 (1000)	1.21	0.265	1.13	0.277
4 (5000)	1.37	0.142	0.95	0.328

Week 13

Group/ dosage (ppm)	Mean hindlimb grip strength (kg)			
	Males	sd	Females	sd
1 (0)	1.34	0.282	1.18	0.390
2 (200)	1.35	0.247	1.10	0.192
3 (1000)	1.36	0.255	1.20	0.314
4 (5000)	1.43	0.175	1.12	0.275

sd Standard deviation

** $p < 0.01$

TABLE 11

Hindlimb splay - group mean values

Pre-dose

Group/ dosage (ppm)	Mean splay values (cm)			
	Males	sd	Females	sd
1 (0)	8.9	1.59	7.6	1.89
2 (200)	9.1	1.82	8.3	2.05
3 (1000)	8.5	1.00	8.4	2.84
4 (5000)	9.5	1.89	8.3	1.85

Week 4

Group/ dosage (ppm)	Mean splay values (cm)			
	Males	sd	Females	sd
1 (0)	9.2	2.00	7.8	2.50
2 (200)	9.5	1.53	7.4	1.05
3 (1000)	9.2	2.02	9.1	2.19
4 (5000)	9.5	2.15	6.4	1.43

Week 8

Group/ dosage (ppm)	Mean splay values (cm)			
	Males	sd	Females	sd
1 (0)	9.3	1.98	8.3	2.62
2 (200)	9.5	1.43	7.9	1.41
3 (1000)	9.1	1.53	8.7	2.37
4 (5000)	10.9	2.38	7.5	2.22

Week 13

Group/ dosage (ppm)	Mean splay values (cm)			
	Males	sd	Females	sd
1 (0)	9.5	2.22	9.1	2.34
2 (200)	9.1	2.00	8.5	1.19
3 (1000)	9.9	2.65	8.9	1.85
4 (5000)	10.5	2.57	7.8	2.54

sd Standard deviation

No statistical significance, $p > 0.05$

TABLE 12

Rectal temperature - group mean values

Pre-dose

Group/ dosage (ppm)	Mean temperature (°C)			
	Males	sd	Females	sd
1 (0)	38.1	0.48	38.2	0.49
2 (200)	38.0	0.54	38.3	0.46
3 (1000)	38.0	0.62	38.3	0.43
4 (5000)	38.1	0.26	38.0	0.34

Week 4

Group/ dosage (ppm)	Mean temperature (°C)			
	Males	sd	Females	sd
1 (0)	38.0	0.46	38.3	0.75
2 (200)	38.2	0.63	38.5	0.47
3 (1000)	38.0	0.56	38.2	0.67
4 (5000)	38.1	0.57	38.5	0.46

Week 8

Group/ dosage (ppm)	Mean temperature (°C)			
	Males	sd	Females	sd
1 (0)	38.1	0.41	38.4	0.60
2 (200)	38.0	0.59	38.4	0.53
3 (1000)	37.7	0.61	38.5	0.59
4 (5000)	37.9	0.61	38.6	0.29

Week 13

Group/ dosage (ppm)	Mean temperature (°C)			
	Males	sd	Females	sd
1 (0)	37.5	0.54	38.1	0.44
2 (200)	37.8	0.59	38.4	0.42
3 (1000)	37.6	0.78	38.3	0.62
4 (5000)	37.7	0.41	38.2	0.54

sd Standard deviation

No statistical significance, $p > 0.05$

TABLE 13

Bodyweights recorded during the functional observational battery - group mean values

Pre-dose

Group/ dosage (ppm)	Mean bodyweights (g)			
	Males	sd	Females	sd
1 (0)	196	7.2	162	10.0
2 (200)	195	6.6	163	7.5
3 (1000)	195	7.6	161	9.4
4 (5000)	195	7.3	168	8.0

Week 4

Group/ dosage (ppm)	Mean bodyweights (g)			
	Males	sd	Females	sd
1 (0)	373	20.1	226	23.1
2 (200)	373	24.4	235	17.3
3 (1000)	369	13.3	222	24.2
4 (5000)	332**	22.8	204*	17.8

Week 8

Group/ dosage (ppm)	Mean bodyweights (g)			
	Males	sd	Females	sd
1 (0)	472	27.4	263	29.0
2 (200)	472	43.7	270	26.7
3 (1000)	469	21.7	252	35.7
4 (5000)	427**	30.2	244	21.5

Week 13

Group/ dosage (ppm)	Mean bodyweights (g)			
	Males	sd	Females	sd
1 (0)	550	33.0	285	38.2
2 (200)	548	53.4	295	32.9
3 (1000)	542	27.9	283	35.0
4 (5000)	480**	44.0	267	27.2

sd Standard deviation

* $p < 0.05$, ** $p < 0.01$

TABLE 14

Locomotor activity - group mean values

Pre-dose

Group/ dosage (ppm)	Mean large movements (in secs) during 1 hour observation period			
	Males	sd	Females	sd
1 (0)	401	75.2	415	99.9
2 (200)	354	148.9	466	129.1
3 (1000)	366	120.7	486	324.0
4 (5000)	338	190.0	460	152.8

Week 4

Group/ dosage (ppm)	Mean large movements (in secs) during 1 hour observation period			
	Males	sd	Females	sd
1 (0)	719	291.3	997	301.3
2 (200)	700	269.1	964	230.4
3 (1000)	698	257.8	1103	379.3
4 (5000)	749	251.5	891	371.4

Week 8

Group/ dosage (ppm)	Mean large movements (in secs) during 1 hour observation period			
	Males	sd	Females	sd
1 (0)	842	295.7	1011	362.0
2 (200)	832	244.0	1021	326.8
3 (1000)	811	172.4	1055	575.1
4 (5000)	781	336.8	931	374.1

Week 13

Group/ dosage (ppm)	Mean large movements (in secs) during 1 hour observation period			
	Males	sd	Females	sd
1 (0)	638	262.1	704	302.0
2 (200)	628	202.9	738	332.3
3 (1000)	558	196.5	738	362.4
4 (5000)	608	276.2	661	310.4

sd Standard deviation

No statistical significance, $p > 0.05$

TABLE 15

Haematology - group mean values

Week 5

Group/ dosage/ ppm	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg
1♂ Control	46.8	15.8	8.26	33.7	56.7	19.1
2♂ 200	46.8	16.1	8.32	34.3	56.2	19.3
3♂ 1000	46.3	16.0	8.22	34.6	56.4	19.5
4♂ 5000	46.6	16.2	8.66	34.7	53.8	18.7
1♀ Control	45.7	16.0	8.22	34.9	55.6	19.4
2♀ 200	44.9	15.8	8.16	35.1	55.1	19.3
3♀ 1000	45.4	15.8	8.14	34.9	55.8	19.5
4♀ 5000	44.1	15.5	7.96	35.1	55.5	19.5

* $p < 0.05$, ** $p < 0.01$

TABLE 15
(Haematology - continued)

Week 5

Group/ dosage/ ppm	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l	LUC 10 ⁹ /l	Plt 10 ⁹ /l	TT s
1♂ Control	15.38	1.78	13.05	0.12	0.06	0.19	0.18	1039	23
2♂ 200	13.54	1.72	11.22	0.11	0.04*	0.23	0.18	1112	23
3♂ 1000	14.27	1.63	12.07	0.11	0.04**	0.20	0.22	1077	22
4♂ 5000	12.46*	1.48	10.55*	0.09	0.04**	0.16	0.14	1079	23
1♀ Control	8.69	1.21	7.12	0.08	0.02	0.15	0.11	931	20
2♀ 200	9.90	1.20	8.33	0.10	0.02	0.14	0.11	1041	19
3♀ 1000	8.59	0.88	7.45	0.06	0.02	0.10	0.08	1063	20
4♀ 5000	7.89	1.02	6.60	0.09	0.02	0.10	0.07	1127**	19

* $p < 0.05$, ** $p < 0.01$

TABLE 15
(Haematology - continued)

Week 13

Group/ dosage/ ppm	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg
1♂ Control	48.8	16.2	9.25	33.2	52.9	17.5
2♂ 200	49.9	16.6	9.49	33.2	52.6	17.5
3♂ 1000	48.3	16.1	9.16	33.4	52.8	17.7
4♂ 5000	48.6	16.3	9.48	33.5	51.3	17.2
1♀ Control	47.7	16.0	8.72	33.5	54.7	18.3
2♀ 200	46.9	15.6	8.51	33.2	55.1	18.3
3♀ 1000	47.5	15.9	8.69	33.6	54.7	18.4
4♀ 5000	**	*	*			
	45.4	15.3	8.31	33.8	54.7	18.5

* $p < 0.05$, ** $p < 0.01$

TABLE 15
(Haematology - continued)

Week 13

Group/ dosage/ ppm	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l	LUC 10 ⁹ /l	Plt 10 ⁹ /l	TT s
1♂ Control	13.41	1.58	11.25	0.16	0.04	0.23	0.15	1082	23
2♂ 200	12.75	1.69	10.53	0.17	0.04	0.20	0.13	1128	24
3♂ 1000	13.62	1.67	11.44	0.17	0.04	0.17	0.13	1148	24
4♂ 5000	13.18	1.72	11.03	0.13	0.04	0.14	0.12	1112	23
1♀ Control	7.32	0.76	6.25	0.11	0.01	0.12	0.06	993	21
2♀ 200	8.20	0.82	7.08	0.10	0.02	0.12	0.06	1045	20
3♀ 1000	8.10	0.89	6.95	0.09	0.02	0.10	0.06	1043	20
4♀ 5000	6.73	0.78	5.70	0.11	0.01	0.09	0.05	1185	19

* $p < 0.05$, ** $p < 0.01$

TABLE 16

Biochemistry - group mean values

Week 5

Group/ dosage/ ppm	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
		Total	Alb	Glob							
1♂ Control	103	6.1	2.9	3.2	14	0.5	291	28	84	<1	3.0
2♂ 200	111	6.1	3.0	3.1	14	0.5	286	29	78	<2	2.3
3♂ 1000	108	6.1	2.9	3.2	13	0.6	271	26	69	<1	3.0
4♂ 5000	104	6.0	2.9	3.1	14	0.5	249	23	66	<2	2.9
1♀ Control	102	6.3	3.1	3.1	15	0.5	186	28	66	<2	3.3
2♀ 200	102	6.5	3.2	3.3	16	0.5	170	30	74	<2	4.0
3♀ 1000	107	6.4	3.2	3.2	15	0.5	175	25	65	<1	2.4
4♀ 5000	105	6.4	**	3.1	13	0.5	150	18	53	<2	2.3

* $p < 0.05$, ** $p < 0.01$

TABLE 16
(Biochemistry - continued)

Week 5

Group/ dosage/ ppm	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	0.2	146	3.9	5.6	4.9	102	78
2♂ 200	0.2	145	3.9	5.4	4.6	103	77
3♂ 1000	0.2	146	3.7	5.4	4.7	103	78
4♂ 5000	0.2	145	3.7	5.4	4.5	103	82
1♀ Control	0.2	145	3.8	5.5	4.0	105	90
2♀ 200	0.2	145	3.6	5.5	3.8	105	92
3♀ 1000	0.2	144	3.6	5.4	3.9	105	77
4♀ 5000	0.2	145	3.5	5.4	3.8	104	93

* $p < 0.05$, ** $p < 0.01$

TABLE 16

(Biochemistry - continued)

Week 13

Group/ dosage/ ppm	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
		Total	Alb	Glob							
1♂ Control	117	6.7	2.9	3.8	14	0.5	193	32	62	<1	4.8
2♂ 200	122	6.5	2.8	3.7	13	0.5	190	32	58	<1	4.0
3♂ 1000	114	6.7	2.8	3.9	14	0.5	156*	31	59	<2	3.7
4♂ 5000	117	6.5	2.7*	3.8	14	0.5	154*	26	54*	<2	4.4
1♀ Control	117	6.6	3.1	3.5	14	0.5	106	49	87	<1	3.9
2♀ 200	123	7.0	3.3	3.7	15	0.5	91	47	76	<1	5.3
3♀ 1000	124	6.8	3.2	3.6	15	0.6	95	26	56	<1	3.9
4♀ 5000	121	7.0*	3.4*	3.6	14	0.5	85	27*	55	<1	6.7

* $p < 0.05$

TABLE 16
(Biochemistry - continued)

Week 13

Group/ dosage/ ppm	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	0.2	145	3.8	5.6	3.9	102	83
2♂ 200	0.2	145	3.9	5.5	3.7	102	77
3♂ 1000	0.2	145	3.9	5.5	3.9	102	85
4♂ 5000	0.2	145	3.7	5.4	3.6	102	85
1♀ Control	0.2	144	4.0	5.4	3.4	103	97
2♀ 200	0.3	144	3.4	5.5	2.9	103	102
3♀ 1000	0.3	144	3.5	5.5	2.8	103	91
4♀ 5000	0.2	143	3.4	5.5	2.5	102	100

* $p < 0.05$, ** $p < 0.01$

TABLE 17

Organ weights - group mean values

Week 14

Group/ dosage/ ppm	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g
Unadjusted means								
1♂ Control	558	2.08	15.1	23.8	1.71	21.4	0.93	3.86
2♂ 200	568	2.11	15.0	22.4	1.70	23.2	0.90	3.74
3♂ 1000	541	2.06	14.2	21.4	1.67	22.2	0.87	3.92
4♂ 5000	485	2.06	14.5	22.3	1.60	21.1	0.79	3.37
Adjusted for bodyweight as covariate								
1♂		-	14.6	23.0	1.67	20.2	0.90	3.74
2♂		-	14.4	21.3	1.64	21.6	0.86	3.57
3♂		-	14.2	21.3	1.66	22.0	0.86	3.90
4♂		-	15.6	24.3	1.70	24.0**	0.85	3.67

** $p < 0.01$

TABLE 17
(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Adrenals	Prostate	Testes		Seminal	Epididymides	
	mg	g	Left g	Right g	Vesicle g	Left g	Right g
Unadjusted means							
1♂ Control	61.0	1.244	1.713	1.727	1.56	0.668	0.707
2♂ 200	58.9	1.195	1.790	1.804	1.38	0.671	0.703
3♂ 1000	61.8	1.193	1.715	1.736	1.46	0.650	0.687
4♂ 5000	57.8	1.126	1.704	1.704	1.32	0.656	0.684

No statistically significant relationship between bodyweight and organ weights
No statistical significance, $p > 0.05$

TABLE 17
(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g	Adrenals mg	Uterus g	Ovaries mg
Unadjusted means											
1♀ Control	271	1.89	15.2	16.6	0.98	11.1	0.56	2.13	72.4	0.56	81.4
2♀ 200	282	1.82	16.2	16.6	1.03	10.9	0.56	2.05	68.1	0.72	79.5
3♀ 1000	281	1.88	15.2	16.1	1.05	11.4	0.59	2.33	71.7	0.81*	78.6
4♀ 5000	267	1.85	17.4	18.0	1.03	11.7	0.53	2.30	64.3	0.87**	89.3
Adjusted for bodyweight as covariate											
1♀		1.89	-	16.8	0.98	11.3	0.57	2.15	72.9	-	-
2♀		1.81	-	16.3	1.02	10.7	0.55	2.03	67.3	-	-
3♀		1.87	-	15.8	1.05	11.2	0.58	2.31	71.1	-	-
4♀		1.86	-	18.4	1.04	12.1	0.54	2.33	65.2	-	-

* $p < 0.05$, ** $p < 0.01$

TABLE 18

Brain parameters - Satellite group

Group/ dosage/ ppm	Terminal bodyweight (g)	Brain measurement		
		Weight (g)	Length (mm)	Width (mm)
1♂ Control	529	1.70	21.0	14.7
2♂ 200	554	1.71	20.9	14.6
3♂ 1000	555	1.71	20.8	14.8
4♂ 5000	477	1.70	21.5	14.5
1♀ Control	314	1.60	20.2	14.2
2♀ 200	309	1.53	20.6	14.2
3♀ 1000	293	1.53	20.0	13.8
4♀ 5000	278	1.57	20.3	14.2

No statistical significance, $p > 0.05$

TABLE 19

Macroscopic incidence summary

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	15 10	15 10	15 10	15 10	15 9	15 10	15 10	15 10
Animals on study Animals completed								
Fur								
Stained - periorbital region/s	0	0	1	0	1	0	0	0
Stained - cranial region	0	0	0	0	0	0	1	1
Badly groomed - dorsum	0	0	0	0	0	0	0	1
Skin								
Right forelimb	0	1	0	0	0	0	0	0
Skin								
Alopecia	1	0	0	0	2	1	0	0
Subcutis								
Haemorrhage	0	0	1	0	1	0	0	0
Eyes								
Damaged	0	0	1	0	1	0	0	0
Tail								
Malaligned	0	0	1	0	0	0	0	0
Incisors								
Pale	0	0	0	0	0	0	0	1
Maloccluded	1	0	0	2	2	0	1	1
Stomach Antrum Mucosa								
White nodule/s	1	3	0	0	2	2	3	4
Adrenals								

TABLE 19
(Macroscopic incidence summary - continued)

Removal reason: Terminal	Group	Group	Group	Group	Group	Group	Group	Group
	1	2	3	4	1	2	3	4
Animals on study Animals completed	----- Males -----				----- Females -----			
	15	15	15	15	15	15	15	15
	10	10	10	10	9	10	10	10
Adrenals Enlarged	(Continued) 0	0	0	0	0	0	1	0
Kidneys Mass	0	0	0	0	0	0	1	0
Cortical depression/s	0	0	0	0	0	0	1	2
Increased pelvic dilatation	0	0	0	0	2	1	2	1
Uterus Fluid distension	0	0	0	0	0	3	5	6
Skeletal Muscle Hindlimbs atrophied	0	0	0	0	0	0	0	1

TABLE 20

Microscopic pathology incidence summary

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
Trachea								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Lungs								
Examined	10	10	10	10	9	10	10	10
No abnormalities detected	7	8	8	8	8	10	9	7
Pneumonitis (Total)	1	1	1	1	1	0	0	1
Minimal	1	1	1	1	1	0	0	1
Increased numbers of alveolar macrophages (Total)	1	0	0	1	0	0	0	0
Minimal	1	0	0	1	0	0	0	0
Subpleural aggregation of alveolar macrophages	1	1	0	0	0	0	0	2
Osteoid deposition (Total)	0	0	1	0	0	0	1	1
Minimal	0	0	1	0	0	0	1	1
Aorta								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Heart								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	3	0	0	5	4	0	0	7
Myocarditis (Total)	4	0	0	1	0	0	0	0
Minimal	2	0	0	0	0	0	0	0
Moderate	2	0	0	1	0	0	0	0
Myocardial fibrosis (Total)	2	0	0	3	4	0	0	2
Minimal	2	0	0	1	4	0	0	1
Moderate	0	0	0	2	0	0	0	1
Mononuclear cells between myocardial fibres (Total)	1	0	0	2	1	0	0	1
Minimal	1	0	0	2	1	0	0	1

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study Animals completed	15 10	15 10	15 10	15 10	15 9	15 10	15 10	15 10
Heart Myocardial degeneration (Total) Minimal	(Continued) 0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0
Thymus Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Lymph Nodes - Cervical Examined No abnormalities detected Increased cellularity - generalised Sinusoidal congestion	10 10 0 0	0 0 0 0	0 0 0 0	10 10 0 0	9 8 1 1	0 0 0 0	1 1 0 0	10 10 0 0
Lymph Nodes - Mesenteric Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	1 1	10 10
Spleen Examined No abnormalities detected Extramedullary haemopoiesis (Total) Minimal	10 5 0 0	0 0 0 0	0 0 0 0	10 6 0 0	9 0 4 4	0 0 0 0	0 0 0 0	10 0 4 4
Haemosiderosis (Total) Minimal Moderate	5 5 0	0 0 0	0 0 0	4 4 0	9 8 1	0 0 0	0 0 0	10 6 4
Liver Examined No abnormalities detected Parenchymal inflammatory cells (Total) Minimal	10 3 0 0	10 0 0 0	10 1 0 0	10 1 1 1	9 3 1 1	10 4 0 0	10 2 0 0	10 4 0 0

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
(Continued)								
Liver	6	10	4	4	1	2	1	2
Centrilobular hepatocyte vacuolation (Total)	5	10	3	3	1	2	1	2
Minimal	1	0	1	1	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Periportal hepatocyte vacuolation (Total)	0	0	0	0	0	1	3	0
Minimal	0	0	0	0	0	1	3	0
Centrilobular hepatocyte hypertrophy (Total)	0	0	2	6	1	0	1	1
Minimal	0	0	2	6	1	0	1	1
Extramedullary haemopoiesis (Total)	3	0	4	2	4	4	4	3
Minimal	3	0	4	2	4	4	4	3
Hepatocyte necrosis (Total)	0	0	0	2	1	0	0	0
Minimal	0	0	0	2	1	0	0	0
Periportal inflammatory cells (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Liver (ORO stain)	10	10	10	10	9	10	10	10
Examined	0	2	0	3	0	0	0	0
No abnormalities detected	10	7	5	7	9	9	9	9
Periportal fat deposition (Total)	0	0	0	0	0	0	0	1
Trace	10	7	5	7	9	8	8	8
Minimal	0	0	0	0	0	1	1	0
Moderate	0	0	0	0	0	1	0	0
Generalised fat deposition (Total)	0	1	1	0	0	1	0	0
Minimal	0	1	1	0	0	1	0	0
Hepatocyte fat - focal (Total)	0	0	4	0	0	0	1	1
Minimal	0	0	4	0	0	0	1	1
Pancreas	10	0	0	10	9	0	0	10
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	0	0	0	0	0	0	0	0
Kidneys	10	10	10	10	9	10	10	10
Examined	8	7	5	8	5	7	5	2
No abnormalities detected	0	0	0	0	0	0	0	0

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
Kidneys	(Continued)							
Pyelitis (Total)	0	0	0	0	0	0	2	0
Moderate	0	0	0	0	0	0	1	0
Severe	0	0	0	0	0	0	1	0
Cortical tubules - basophilic (Total)	0	2	4	1	2	0	1	6
Minimal	0	2	2	1	2	0	1	5
Moderate	0	0	2	0	0	0	0	1
Tubular dilatation (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Dystrophic mineralisation (Total)	0	0	0	0	2	2	2	6
Minimal	0	0	0	0	2	2	1	3
Moderate	0	0	0	0	0	0	1	3
Dilatation of the renal pelvis (Total)	0	0	0	1	2	1	2	1
Minimal	0	0	0	1	2	1	2	0
Moderate	0	0	0	0	0	0	0	1
Interstitial inflammatory cells (Total)	0	0	1	0	0	0	1	2
Minimal	0	0	1	0	0	0	0	1
Moderate	0	0	0	0	0	0	1	1
Severe	0	0	0	0	0	0	0	0
Medullary cyst	1	0	0	0	0	0	0	1
Cystic cortical tubules (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Pelvic transitional cell hyperplasia (Total)	0	1	0	0	0	0	0	0
Minimal	0	1	0	0	0	0	1	0
Severe	0	0	0	0	0	0	0	0
Peripelvic lymphoid aggregations (Total)	0	0	0	0	0	0	1	0
Marked	0	0	0	0	0	0	1	0
Malignant lymphoma	0	0	0	0	0	0	1	0

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
	----- Males -----				----- Females -----			
Kidneys	(Continued)	0	0	0	0	0	0	1
Pelvic urolithiasis								
Urinary Bladder								
Examined	10	0	0	0	9	0	0	10
No abnormalities detected	9	0	0	10	9	0	0	10
Subepithelial inflammatory cells (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Uterus								
Examined	0	0	0	0	9	3	5	10
No abnormalities detected	0	0	0	0	9	0	0	4
Luminal dilatation (Total)	0	0	0	0	0	3	5	6
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	3	5	5
Endometrial polypoid hyperplasia (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Cervix								
Examined	0	0	0	0	9	0	0	10
No abnormalities detected	0	0	0	0	9	0	0	9
Squamous epithelial hyperplasia	0	0	0	0	0	0	0	1
Squamous epithelial hyperkeratosis	0	0	0	0	0	0	0	1
Ovaries								
Examined	0	0	0	0	9	0	0	10
No abnormalities detected	0	0	0	0	9	0	0	10
Prostate								
Examined	10	0	0	0	0	0	0	0
No abnormalities detected	4	0	0	7	0	0	0	0

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
Prostate	(Continued)							
Interstitial inflammation (Total)	6	0	0	3	0	0	0	0
Minimal	5	0	0	3	0	0	0	0
Moderate	1	0	0	0	0	0	0	0
Seminal Vesicles								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
Epididymides								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
Testes								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
Almost total depletion of germ cells leaving only Sertoli cells (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Thyroids								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	6	0	0	4	9	0	0	10
Follicular cell hypertrophy (Total)	4	0	0	4	0	0	0	0
Minimal	4	0	0	4	0	0	0	0
Ectopic thymus	1	0	0	2	0	0	0	0
Parathyroids								
Examined	9	0	0	8	8	0	0	10
Missing	1	0	0	2	1	0	0	0
No abnormalities detected	9	0	0	8	8	0	0	10
Adrenals								
Examined	10	0	0	10	9	0	1	10
No abnormalities detected	8	0	0	7	9	0	1	10

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
(Continued)								
Adrenals								
Diffuse cellular vacuolation - zona fasciculata (Total)	2	0	0	3	0	0	0	0
Minimal	0	0	0	3	0	0	0	0
Moderate	2	0	0	0	0	0	0	0
Pituitary								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	4	0	0	6	9	0	0	10
Cyst(s) in pars anterior	2	0	0	1	0	0	0	0
Focal vacuolation - pars distalis	5	0	0	3	0	0	0	0
Salivary Glands								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Oesophagus								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Stomach								
Examined	10	3	0	10	9	2	3	10
No abnormalities detected	9	0	0	9	7	0	1	6
Ectopic non-glandular epithelium within the glandular mucosa	1	3	0	1	2	2	2	4
Duodenum								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Jejunum								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10
Ileum								
Examined	10	0	0	10	9	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study Animals completed	15 10	15 10	15 10	15 10	15 9	15 10	15 10	15 10
Caecum Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Colon Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Rectum Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Bone Marrow/sternum Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Brain Examined No abnormalities detected	10 10	0 0	0 0	10 10	9 9	0 0	0 0	10 10
Sciatic Nerve Examined No abnormalities detected Degenerate fibres (Total) Minimal	10 9 1 1	0 0 0 0	0 0 0 0	10 10 0 0	9 9 0 0	0 0 0 0	0 0 0 0	10 10 0 0
Eyes Examined Panophthalmitis	0 0	0 0	1 1	0 0	1 1	0 0	0 0	0 0
Skeletal Muscle Examined	0	0	0	0	0	0	0	1

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Main	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	9	10	10	10
Skeletal Muscle	(Continued)							
Myofibre atrophy (Total)	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Myofibre replacement by adipose tissue (Total)	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	0	1
Skin								
Examined	0	1	0	0	0	0	0	0
Scab	0	1	0	0	0	0	0	0
Epidermal hyperplasia (Total)	0	1	0	0	0	0	0	0
Moderate	0	1	0	0	0	0	0	0
Dermal inflammation (Total)	0	1	0	0	0	0	0	0
Marked	0	1	0	0	0	0	0	0
Subcutis								
Examined	0	0	1	0	1	0	0	0
Haemorrhage and inflammation-periorbital region	0	0	1	0	1	0	0	0
Scab and epidermal hyperplasia - periorbital region	0	0	1	0	0	0	0	0
Spinal Cord								
Examined	0	0	0	0	0	0	0	1
No abnormalities detected	0	0	0	0	0	0	0	1
Lymphoreticular Tumour								
Examined	0	0	0	0	0	0	1	0
Malignant lymphoma (Malignant)	0	0	0	0	0	0	1	0

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Satellite	Group	Group	Group	Group	Group	Group	Group	Group
	1	2	3	4	1	2	3	4
Animals on study Animals completed	15	15	15	15	15	15	15	15
	5	0	0	5	5	0	0	5
----- Males -----								
----- Females -----								
Forebrain/cerebrum	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Midbrain	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Cerebellum And Pons	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Medulla Oblongata	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Spinal Cord (C3-6)	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Spinal Cord (L1-4)	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Gasserian Ganglia	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Dorsal Root Ganglion (C)	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
Dorsal Root Ganglion (L)	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								

TABLE 20
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal Satellite	Group	Group	Group	Group	Group	Group	Group	Group
	1	2	3	4	1	2	3	4
Animals on study								
Animals completed	15	15	15	15	15	15	15	15
	5	0	0	5	5	0	0	5
Dorsal Root Fibres (C)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Dorsal Root Fibres (L)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Ventral Root Fibres (C)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Ventral Root Fibres (L)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Sciatic Nerve (Sciatic Notch)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Sciatic Nerve (Mid-thigh)								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Sural Nerve								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
Tibial Nerve								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5

APPENDIX 1

Bodyweights - individual values (g) - Main group

Group 1♂ Control

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	204	274	341	387	427	466	488	515	527	554	574	592	607	620	622
2	201	264	317	353	395	419	433	450	462	474	500	510	520	532	527
3	194	257	302	347	381	412	416	443	458	469	477	493	504	508	500
4	202	271	331	372	415	444	468	500	515	530	547	561	573	575	569
5	200	269	325	363	399	422	439	471	494	517	541	561	578	593	576
6	197	255	302	345	388	419	445	472	491	520	540	552	571	580	573
7	192	247	293	335	370	396	438	444	477	484	509	527	546	558	553
8	186	254	314	357	394	421	454	474	514	521	549	576	594	608	596
9	196	252	307	355	400	435	456	488	511	526	544	555	571	587	583
10	190	248	306	349	379	407	424	462	488	500	528	539	566	572	563

Group 2♂ 200 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
11	195	266	318	326	413	447	463	485	511	518	547	559	582	578	570
12	198	264	313	369	411	445	464	497	506	525	545	561	578	596	583
13	202	269	325	374	411	446	471	499	527	554	579	588	602	617	613
14	206	280	339	398	459	494	511	547	567	592	622	634	654	671	677
15	193	259	308	356	398	424	447	484	504	518	539	559	570	571	560
16	201	261	315	356	395	429	440	476	499	514	531	544	556	567	560
17	190	252	314	345	402	437	462	502	530	552	569	594	614	636	627
18	196	244	286	319	348	371	388	405	417	435	444	455	466	468	466
19	188	240	283	329	365	382	405	441	459	481	500	505	521	533	519
20	198	259	316	366	409	438	453	493	521	537	559	572	592	601	588

APPENDIX 1

(Bodyweights - continued)

Group 3♂ 1000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
21	205	265	319	366	396	428	448	471	489	505	481	504	526	530	525
22	197	258	314	351	404	440	458	488	508	527	546	565	576	588	579
23	202	259	327	372	422	445	463	492	511	528	542	553	556	567	546
24	199	260	319	374	411	439	449	482	505	514	545	567	584	597	594
25	196	269	322	371	397	432	453	478	505	516	542	555	575	589	591
26	192	253	292	332	372	395	415	443	474	488	499	512	521	536	527
27	200	258	309	350	383	417	438	474	499	524	547	561	574	587	585
28	185	255	317	361	399	415	430	453	471	495	505	518	535	545	536
29	188	255	288	326	354	390	393	419	443	457	473	481	488	502	490
30	194	248	301	338	377	400	416	441	460	483	499	518	528	536	536

Group 4♂ 5000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
31	207	262	309	334	358	386	400	421	432	447	469	471	480	479	486
32	195	252	287	308	330	351	370	385	399	402	418	421	432	443	438
33	202	283	320	342	350	370	386	419	444	469	497	517	538	544	543
34	189	247	290	327	353	367	379	409	415	427	439	448	461	470	470
35	195	257	296	323	319	341	357	396	413	431	455	461	484	497	487
36	199	260	310	328	347	364	391	415	448	464	472	497	505	524	516
37	200	255	282	297	302	329	342	365	392	411	431	436	456	461	449
38	193	248	286	308	323	331	345	378	399	422	432	441	456	467	467
39	189	246	288	311	323	344	362	394	416	441	458	462	474	486	479
40	197	254	307	342	364	386	416	452	483	513	543	546	553	565	565

APPENDIX 1

(Bodyweights - continued)

Group 1♀ Control

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
41	159	192	211	231	235	244	246	254	258	263	273	275	282	278	262
42	156	183	197	212	229	245	244	251	262	269	280	280	283	290	282
43	148	187	210	228	242	255	265	275	278	291	306	302	302	315	306
44	158	199	219	232	249	261	264	277	284	295	296	305	308	317	304
45	156	185	209	227	245	256	261	274	280	280	276	283	288	285	272
46	152	180	202	226	233	244	254	260	267	266	273	284	278	279	279
47	146	171	179	193	204	204									
48	142	169	182	202	213	222	224	237	249	242	240	251	262	270	265
49	151	169	187	203	214	214	220	228	231	240	239	243	249	248	236
50	145	162	180	192	200	201	209	217	222	218	226	233	235	237	237

Group 2♀ 200 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
51	149	175	194	204	215	219	231	238	243	245	246	255	250	262	260
52	153	181	199	212	226	230	233	240	240	245	250	258	257	257	259
53	157	196	217	231	240	260	261	278	277	288	290	298	303	320	307
54	159	189	212	228	246	255	257	268	275	280	286	293	298	302	297
55	146	175	201	222	232	236	244	252	258	261	266	271	276	275	279
56	156	196	217	233	257	270	274	285	295	311	314	324	336	346	335
57	144	175	201	215	233	238	240	253	259	265	267	277	284	280	272
58	151	194	217	236	254	257	269	276	282	290	283	300	302	312	302
59	143	170	187	198	213	220	232	246	252	250	253	263	259	262	262
60	153	181	203	223	234	241	244	258	263	256	265	278	275	282	279

No. 47 died following blood sample procedures during Week 5

APPENDIX 1

(Bodyweights - continued)

Group 3♀ 1000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
61	163	207	239	264	292	314	320	336	337	348	367	376	380	388	381
62	152	173	192	194	209	209	222	230	244	241	246	258	270	267	272
63	161	198	211	223	233	248	257	263	265	273	284	291	287	288	291
64	154	186	209	221	240	254	252	262	264	271	276	281	294	297	290
65	151	175	197	207	220	231	242	248	249	260	271	271	278	284	283
66	146	168	177	201	216	214	217	232	240	241	240	250	256	260	255
67	156	186	209	223	238	253	265	271	278	290	291	308	306	317	309
68	145	167	182	185	198	198	197	199	206	210	209	214	222	226	220
69	140	165	184	204	218	223	222	235	237	241	246	258	266	267	257
70	149	183	202	215	232	238	244	255	261	265	272	269	276	280	275

Group 4♀ 5000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
71	159	195	203	197	198	207	221	238	238	248	253	259	261	263	263
72	150	186	191	196	194	199	210	222	223	221	234	240	237	239	238
73	156	190	201	201	210	223	222	231	244	250	255	258	265	278	265
74	144	186	197	201	214	223	222	244	259	262	272	282	281	286	282
75	150	190	203	217	219	237	234	250	258	267	287	287	306	308	310
76	154	185	203	175	193	213	223	239	237	242	249	267	269	281	275
77	156	196	212	220	221	228	239	246	255	264	268	279	278	293	292
78	147	184	200	214	213	222	229	238	242	255	266	268	265	268	264
79	144	182	194	190	187	202	220	236	240	260	264	265	265	272	282
80	151	179	198	199	199	202	215	230	230	223	233	241	244	242	240

APPENDIX 1

(Bodyweights - continued - Satellite group)

Group 1♂ Control

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
81	193	254	321	369	408	435	468	504	518	529	537	551	559	565	577
82	191	246	292	311	341	362	389	414	437	454	469	486	501	507	511
83	197	259	310	360	385	401	433	443	470	495	507	521	534	540	539
84	189	245	300	341	383	403	432	455	463	472	480	499	512	518	522
85	185	258	304	348	378	397	410	440	464	471	495	501	514	522	520

Group 2♂ 200 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
86	187	239	279	310	336	354	374	373	390	410	429	443	451	457	453
87	188	250	313	364	410	432	463	493	519	533	549	564	576	600	612
88	195	249	304	312	369	406	431	464	485	496	521	539	551	550	569
89	200	261	308	345	379	394	427	463	490	510	531	563	570	589	591
90	192	254	308	356	395	422	444	488	503	516	539	555	572	578	579

Group 3♂ 1000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
91	196	247	301	334	367	385	406	427	441	457	467	464	494	507	508
92	195	258	309	348	383	404	433	463	485	505	525	553	573	588	593
93	188	244	283	312	349	384	419	451	471	495	518	539	563	578	578
94	192	259	319	364	397	425	448	478	508	521	540	546	553	564	558
95	187	255	316	353	395	424	447	474	497	504	516	539	542	557	560

Group 4♂ 5000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
96	188	251	293	333	372	402	423	444	463	481	425	443	470	485	490
97	188	248	281	307	320	344	367	384	418	424	396	418	439	458	455
98	195	259	301	347	368	392	417	450	469	487	497	512	532	538	545
99	196	254	300	340	354	364	394	417	433	447	463	479	493	496	503
100	192	251	278	318	340	362	379	408	423	396	419	422	412	413	420

APPENDIX 1

(Bodyweights - continued)

Group 1♀ Control

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
101	160	192	214	231	249	259	268	278	276	284	290	296	297	301	303
102	155	188	219	234	241	263	275	283	280	296	308	317	317	323	325
103	166	195	222	242	264	282	292	305	313	318	327	339	350	353	348
104	155	177	199	219	238	243	248	260	262	266	269	279	281	279	279
105	148	175	201	220	236	256	268	277	279	289	301	311	310	313	320

Group 2♀ 200 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
106	149	172	193	206	216	211	219	229	227	225	225	237	234	241	245
107	152	178	206	230	235	246	255	271	271	287	288	297	301	303	299
108	162	185	212	242	256	271	287	307	309	317	330	340	343	350	360
109	167	193	219	243	256	260	279	295	300	301	313	321	319	321	326
110	156	186	205	226	245	253	265	277	282	285	299	302	308	312	309

Group 3♀ 1000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
111	164	203	226	253	264	275	286	303	313	309	311	330	332	330	329
112	158	181	198	211	222	231	239	242	252	248	255	258	264	260	265
113	153	180	214	243	265	274	296	314	318	315	324	338	342	344	348
114	155	179	181	192	201	210	213	214	223	235	238	239	241	245	241
115	149	177	192	199	212	223	230	236	245	252	257	261	270	274	274

Group 4♀ 5000 ppm

Animal number	Week														
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
116	150	175	180	188	193	197	204	202	209	214	216	215	218	223	218
117	151	182	196	203	204	205	225	240	244	252	262	267	266	262	267
118	163	202	224	237	238	252	267	281	282	290	306	312	320	325	322
119	163	197	220	222	222	227	237	258	259	261	267	277	273	271	279
120	157	190	208	213	214	233	245	255	263	277	276	285	293	300	297

APPENDIX 2

Food consumption - individual values (g/rat/week)

Group 1♂ Control

Week	Animal									
	1	2	3	4	5	6	7	8	9	10
-1	(3)	196	200	208	227	218	191	199	197	214
1	256	219	222	237	248	248	229	232	226	240
2	251	210	217	229	236	246	217	225	228	238
3	236	224	211	228	256	267	228	242	244	245
4	245	225	222	232	238	263	222	235	240	233
5	226	193	197	208	223	231	216	217	226	211
6	244	215	204	225	245	256	224	267	238	253
7	223	197	199	215	242	259	219	245	227	239
8	231	200	206	217	238	245	213	236	234	226
9	232	217	200	218	244	257	220	237	228	246
10	229	205	206	221	242	246	229	231	216	242
11	226	214	208	212	236	250	217	235	224	248
12	234	209	205	209	227	239	222	238	221	234
13	214	191	208	193	209	198	184	215	201	195

- () Value excluded from means and statistical analysis - exceedingly low value, weight gain appeared normal

APPENDIX 2

(Food consumption - continued)

Group 2♂ 200 ppm

Week	Animal									
	11	12	13	14	15	16	17	18	19	20
- 1	201	208	213	216	206	202	193	181	176	212
1	225	228	238	252	227	226	226	208	208	253
2	243	247	242	285	243	223	205	197	213	241
3	251	252	244	298	249	239	257	202	226	253
4	259	262	253	295	252	239	256	206	210	253
5	222	221	236	236	225	215	240	185	201	232
6	232	251	247	268	263	230	261	201	233	258
7	238	225	241	246	247	221	249	182	197	273
8	224	241	255	238	261	239	240	190	226	251
9	246	241	249	236	252	233	253	191	219	254
10	236	246	234	245	249	227	239	186	211	245
11	233	245	230	239	241	213	255	178	208	243
12	225	251	230	241	234	223	246	182	209	237
13	209	212	206	224	203	211	221	171	191	211

APPENDIX 2

(Food consumption - continued)

Group 3♂ 1000 ppm

Week	Animal									
	21	22	23	24	25	26	27	28	29	30
-1	203	202	188	200	227	198	197	207	191	187
1	231	222	228	240	265	211	213	250	201	213
2	239	241	233	253	274	207	208	240	204	220
3	252	247	252	253	261	232	224	235	212	240
4	254	253	253	254	258	244	234	227	227	235
5	215	235	210	206	235	218	208	217	196	204
6	222	236	241	228	256	224	234	233	224	220
7	229	230	237	222	248	223	229	224	216	212
8	225	231	238	247	258	220	226	233	208	221
9	194	232	228	235	247	208	222	233	210	228
10	180	236	220	235	250	201	212	230	190	224
11	221	224	222	239	256	203	214	223	190	219
12	212	226	209	228	233	209	213	222	187	216
13	202	207	181	216	230	205	203	206	170	206

APPENDIX 2

(Food consumption - continued)

Group 4♂ 5000 ppm

Week	Animal									
	31	32	33	34	35	36	37	38	39	40
- 1	(18)	189	221	188	198	209	193	186	201	208
1	201	192	241	181	223	241	193	204	207	215
2	209	183	230	204	227	235	206	199	216	231
3	208	192	210	207	187	222	200	196	224	245
4	220	195	223	201	175	221	212	199	217	242
5	198	183	199	193	157	195	186	186	204	233
6	204	195	237	201	203	229	206	212	226	257
7	195	170	221	184	192	224	199	203	215	261
8	199	168	227	189	210	217	189	206	227	268
9	200	178	229	181	213	220	197	196	220	271
10	191	170	229	172	204	216	185	188	201	235
11	191	183	225	178	213	210	192	186	210	234
12	187	179	205	179	212	216	186	194	194	238
13	186	163	196	182	195	202	174	181	187	233

() Value excluded from means and statistical analysis - exceedingly low value, weight gain appeared normal

APPENDIX 2

(Food consumption - continued)

Group 1 ♀ Control

Week	Animal									
	41	42	43	44	45	46	47	48	49	50
- 1	152	147	142	163	148	144	143	143	133	138
1	180	151	150	167	161	165	139	147	146	149
2	164	161	159	165	164	153	140	151	142	145
3	156	171	160	176	171	162	136	151	145	150
4	163	173	172	183	175	159	136	159	140	146
5	147	146	144	150	146	161		135	125	126
6	145	156	155	164	154	146		145	141	131
7	145	154	150	163	155	147		144	132	131
8	150	161	163	163	145	132		143	131	140
9	143	146	159	148	132	139		125	138	133
10	141	147	151	152	149	135		137	129	133
11	142	143	145	153	142	130		145	126	126
12	141	137	151	147	132	134		140	118	128
13	106	128	134	134	120	131		131	112	122

No. 47 died following blood sampling procedures during Week 5

APPENDIX 2

(Food consumption - continued)

Group 2♀ 200 ppm

Week	Animal									
	51	52	53	54	55	56	57	58	59	60
- 1	135	142	162	152	109	162	142	170	137	147
1	148	153	165	159	151	169	164	187	150	166
2	145	147	159	173	160	170	163	189	152	160
3	149	152	163	170	165	183	161	196	155	162
4	158	154	176	179	186	177	157	191	157	158
5	143	127	147	142	143	145	140	178	148	143
6	135	129	161	146	144	165	150	174	154	155
7	141	125	152	153	148	167	147	179	151	145
8	138	132	156	152	148	164	140	182	147	138
9	121	122	142	146	144	157	142	150	141	145
10	138	124	160	147	134	145	172	186	146	144
11	124	116	149	145	142	169	132	174	136	134
12	138	121	162	143	152	155	133	181	148	142
13	128	111	140	130	142	130	122	159	140	134

APPENDIX 2

(Food consumption - continued)

Group 3 ♀ 1000 ppm

Week	Animal									
	61	62	63	64	65	66	67	68	69	70
-1	179	140	156	157	138	132	144	141	144	149
1	193	146	159	161	169	142	160	145	157	165
2	202	140	155	161	143	149	164	149	161	164
3	223	142	158	172	152	148	174	154	157	175
4	228	152	166	175	169	137	174	135	154	161
5	176	126	147	144	142	134	140	131	141	153
6	191	132	146	150	138	149	160	125	148	163
7	184	137	145	130	137	143	166	151	138	162
8	182	132	147	157	148	134	180	126	143	152
9	172	130	140	150	151	134	163	123	144	142
10	187	132	139	152	137	139	166	129	147	145
11	179	128	140	151	137	137	169	129	148	149
12	171	135	146	156	139	137	157	126	139	150
13	143	131	137	130	132	124	144	114	131	127

APPENDIX 2

(Food consumption - continued)

Group 4♀ 5000 ppm

Week	Animal									
	71	72	73	74	75	76	77	78	79	80
- 1	152	152	151	144	160	142	164	150	163	146
1	145	143	128	142	145	142	170	148	158	151
2	141	145	133	146	165	117	165	151	151	156
3	143	136	132	149	162	139	165	153	152	142
4	152	137	136	157	175	182	162	149	159	141
5	139	126	131	138	148	165	144	130	149	125
6	144	124	132	151	163	181	154	151	165	136
7	144	123	140	146	158	167	156	148	163	126
8	140	129	139	150	166	163	160	148	167	114
9	134	124	126	146	162	164	149	142	158	133
10	129	124	129	140	153	163	151	138	152	121
11	133	117	130	131	167	163	152	137	153	118
12	134	123	143	144	173	153	166	141	151	129
13	128	116	122	126	151	135	136	119	139	118

APPENDIX 2

(Food consumption - continued - Satellite group)

Week	Group 1♂ Control					Group 2♂ 200 ppm				
	Animal									
	81	82	83	84	85	86	87	88	89	90
- 1	188	199	190	189	206	192	212	187	208	189
1	236	221	219	226	233	203	262	215	243	235
2	234	202	221	220	232	218	282	187	240	228
3	240	196	218	230	222	215	277	238	232	238
4	234	194	221	230	213	218	274	241	221	244
5	240	204	229	219	226	214	277	229	234	220
6	231	199	212	213	221	200	260	225	233	225
7	(258)	207	206	217	222	177	267	213	231	224
8	217	201	214	215	230	198	259	205	216	220
9	215	215	224	204	222	215	263	222	238	219
10	210	204	224	204	206	217	250	213	234	225
11	222	207	226	209	216	196	254	215	232	220
12	214	209	219	208	206	191	240	212	237	221
13	206	198	194	212	206	173	243	214	220	219

- () Value excluded from means and statistical analysis - very high residue on one day of week - only 0.5 g consumed

APPENDIX 2

(Food consumption - continued)

Week	Group 3♂ 1000 ppm					Group 4♂ 5000 ppm				
	Animal									
	91	92	93	94	95	96	97	98	99	100
- 1	186	212	186	203	200	180	184	199	191	178
1	218	232	201	235	236	196	191	209	204	170
2	213	230	195	228	229	202	192	230	212	187
3	215	250	198	228	236	208	208	228	209	202
4	208	244	215	220	230	211	200	221	216	194
5	201	238	224	233	221	209	205	219	209	202
6	197	245	223	220	217	204	199	222	200	194
7	181	255	209	229	219	203	203	216	201	195
8	187	246	212	216	210	199	190	198	198	164
9	193	243	221	227	205	134	159	207	200	166
10	164	250	211	209	213	127	140	207	197	160
11	196	269	215	191	205	167	185	219	198	147
12	203	261	217	202	213	176	198	213	192	131
13	191	239	209	206	199	178	194	206	198	173

APPENDIX 2

(Food consumption - continued)

Week	Group 1 ♀ Control					Group 2 ♀ 200 ppm				
	Animal									
	101	102	103	104	105	106	107	108	109	110
- 1	155	143	156	134	144	135	143	144	149	146
1	166	170	157	150	134	155	173	163	167	154
2	167	164	166	157	147	140	166	174	169	163
3	171	165	180	162	165	147	161	172	176	174
4	177	182	182	154	167	144	171	186	182	171
5	164	162	168	147	160	129	164	173	184	167
6	152	165	172	155	165	131	168	175	169	162
7	149	150	168	138	157	126	144	169	158	157
8	150	158	152	138	149	115	151	162	162	143
9	153	160	158	142	155	116	150	173	173	156
10	140	150	159	142	152	122	154	159	160	155
11	147	154	163	144	149	125	150	163	144	155
12	149	154	158	131	155	125	147	164	157	158
13	146	139	141	128	140	135	148	167	162	141

APPENDIX 2

(Food consumption - continued)

Week	Group 3♂ 1000 ppm					Group 4♂ 5000 ppm				
	Animal									
	111	112	113	114	115	116	117	118	119	120
- 1	164	148	149	143	154	143	139	173	152	142
1	177	150	163	135	151	138	137	155	162	159
2	182	156	179	136	154	150	135	176	154	151
3	183	168	196	142	160	143	133	173	151	154
4	186	170	197	139	155	138	139	181	159	159
5	182	153	196	129	153	130	132	168	142	156
6	177	159	191	128	160	127	136	167	142	161
7	173	149	178	131	150	129	128	154	137	146
8	154	143	166	130	141	122	134	155	131	146
9	153	146	150	133	145	127	134	157	139	150
10	170	138	183	119	146	118	120	155	128	142
11	161	139	172	125	145	119	127	148	125	150
12	154	138	162	123	147	113	121	145	131	145
13	157	131	165	119	134	112	129	146	130	139

APPENDIX 3

Water consumption - individual values (g/rat/day) - Main group

Group 1♂ Control

Week 12	Animal									
	1	2	3	4	5	6	7	8	9	10
Day										
1	43	33	32	43	39	41	29	45	31	30
2	45	36	45	46	48	40	34	47	33	41
3	42	36	35	42	41	41	35	48	39	37
4	56	33	36	48	43	40	39	50	33	37
5	45	32	39	34	44	39	34	49	35	34
6	39	37	35	44	43	32	37	42	35	40
7	39	40	32	36	45	38	38	48	33	36

Group 2♂ 200 ppm

Week 12	Animal									
	11	12	13	14	15	16	17	18	19	20
Day										
1	56	34	30	32	33	43	39	25	31	41
2	60	41	39	35	38	44	38	31	34	43
3	64	40	39	33	33	58	36	28	38	44
4	60	41	39	31	34	52	45	30	32	36
5	67	38	36	32	33	50	39	28	41	40
6	59	39	34	30	35	54	42	27	37	41
7	57	40	38	31	33	46	37	30	37	43

APPENDIX 3

(Water consumption - continued)

Group 3♂ 1000 ppm

Week 12	Animal									
	21	22	23	24	25	26	27	28	29	30
Day										
1	36	41	32	34	36	38	30	43	29	35
2	41	44	44	41	5	35	31	45	26	35
3	43	43	33	42	58	37	29	40	24	30
4	48	40	42	39	32	37	37	44	30	36
5	38	39	36	43	35	41	33	41	23	30
6	43	37	41	40	43	35	35	43	26	38
7	44	40	41	41	38	42	34	41	29	33

Group 4♂ 5000 ppm

Week 12	Animal									
	31	32	33	34	35	36	37	38	39	40
1	53	27	29	32	41	26	45	48	28	35
2	52	29	34	45	46	40	53	47	43	44
3	49	29	38	38	38	36	58	51	34	28
4	53	28	38	39	45	34	59	47	34	40
5	42	28	34	43	38	38	48	46	44	32
6	49	28	32	41	39	32	52	52	35	44
7	45	31	35	45	49	41	55	44	35	38

APPENDIX 3

(Water consumption - continued)

Group 1 ♀ Control

Week 12	Animal									
	41	42	43	44	45	46	47	48	49	50
Day										
1	27	22	35	24	23	25		23	25	21
2	33	23	48	32	21	32		22	23	22
3	35	23	33	33	26	34		31	26	18
4	34	21	38	35	32	32		27	22	23
5	41	18	41	26	22	29		26	21	22
6	33	25	33	33	26	30		17	22	20
7	26	23	39	30	23	22		32	23	22

Group 2 ♀ 200 ppm

Week 12	Animal									
	51	52	53	54	55	56	57	58	59	60
Day										
1	26	30	42	35	24	24	29	40	26	23
2	34	25	40	29	23	30	26	44	21	24
3	25	25	74	35	23	28	31	45	23	18
4	31	27	31	41	24	28	30	46	23	22
5	23	26	25	35	23	23	26	50	27	29
6	31	31	61	29	23	31	27	39	22	24
7	23	23	55	28	20	33	27	36	20	19

APPENDIX 3

(Water consumption - continued)

Group 3♀ 1000 ppm

Week 12	Animal									
	61	62	63	64	65	66	67	68	69	70
Day										
1	31	27	29	28	32	37	39	18	26	30
2	37	22	31	27	36	31	36	21	24	39
3	35	20	28	29	36	36	40	25	29	46
4	32	26	26	26	38	38	39	20	31	29
5	33	27	31	23	36	39	35	17	25	29
6	37	21	33	25	33	33	37	22	22	34
7	31	19	29	26	56	36	37	22	31	37

Group 4♀ 5000 ppm

Week 12	Animal									
	71	72	73	74	75	76	77	78	79	80
Day										
1	24	26	20	25	42	36	46	20	29	25
2	27	29	31	23	39	46	46	26	31	28
3	24	20	32	27	42	38	31	23	25	21
4	27	25	29	29	50	40	36	23	23	25
5	27	22	21	26	44	41	45	24	27	23
6	25	25	31	23	47	33	40	18	34	25
7	22	19	29	22	41	41	33	20	24	22

APPENDIX 3

(Water consumption - continued - Satellite group)

Group 1♂
Control

Week 12	Animal				
	81	82	83	84	85
Day					
1	52	40	33	27	49
2	43	49	33	43	66
3	39	49	35	39	50
4	34	47	39	43	47
5	48	48	38	42	50
6	34	41	35	32	68
7	41	43	30	40	50

Group 2♂
200 ppm

Week 12	Animal				
	86	87	88	89	90
Day					
1	32	44	34	33	41
2	33	36	38	38	44
3	28	42	38	37	41
4	34	42	32	31	43
5	33	42	37	35	44
6	37	41	38	39	41
7	37	44	35	38	39

Group 3♂
1000 ppm

Week 12	Animal				
	91	92	93	94	95
Day					
1	25	41	30	39	42
2	30	43	28	40	45
3	28	46	33	36	46
4	32	43	28	40	47
5	29	45	29	40	46
6	25	40	33	44	43
7	29	38	27	44	45

Group 4♂
5000 ppm

Week 12	Animal				
	96	97	98	99	100
Day					
1	34	45	36	33	60
2	39	46	38	38	41
3	35	38	40	36	37
4	36	51	42	34	36
5	36	49	33	37	36
6	36	50	39	34	35
7	36	49	40	41	32

APPENDIX 3

(Water consumption - continued)

Group 1 ♀
Control

Week 12	Animal				
	101	102	103	104	105
Day					
1	35	33	33	20	37
2	34	38	34	19	38
3	34	34	30	23	38
4	29	32	33	23	41
5	33	36	35	19	33
6	34	31	33	17	38
7	35	31	33	21	36

Group 2 ♀
200 ppm

Week 12	Animal				
	106	107	108	109	110
Day					
1	25	32	32	37	24
2	23	26	30	33	28
3	29	36	30	23	25
4	29	33	32	38	28
5	24	39	38	32	22
6	23	31	32	29	29
7	26	30	30	25	26

Group 3 ♀
1000 ppm

Week 12	Animal				
	111	112	113	114	115
Day					
1	31	35	29	19	30
2	25	40	36	23	31
3	33	38	36	26	31
4	35	40	32	21	35
5	32	41	42	20	29
6	26	37	41	20	29
7	37	36	36	23	34

Group 4 ♀
5000 ppm

Week 12	Animal				
	116	117	118	119	120
Day					
1	21	23	23	22	25
2	20	24	22	29	32
3	24	20	29	24	29
4	24	27	28	24	31
5	18	31	25	30	32
6	20	20	29	23	32
7	24	21	29	(172)	37

() Value excluded from means and statistical analysis, leakage

APPENDIX 4

Functional observational battery

KEY

Tremors

B Body

blank no tremor observed

the numbers associated with tremors indicate the degree of effect
1,2,3 increasing degree of effect

Posture

S Sitting/standing in cage

R Rearing in cage

Ease of removal from cage

2 easy (little resistance)

3 moderately easy

5 difficult (noticeable squirming or twisting making handling difficult)

Ease of handling

2 easy (little resistance)

3 moderately difficult

5 difficult (runs around cage, or is hard to grab - noticeable)

Salivation (only scored if present)

Y sign observed with 1 being slight

N sign not observed

Arousal

2,3,4,5 increasing levels of arousal with 4 being alert

Gait

T Walking on toes

HU Hunched

A Swaying/lurching gait

O Unusual gait - see additional comments

U Unable to assess - see additional comments

blank normal gait

the numbers associated with gait indicate the degree of effect
1,2,3 increasing degree of effect

APPENDIX 4

(Functional observational battery - continued)

Approach response

- 1 no reaction
- 2 sniffs only
- 3 approaches **and** sniffs
- 4 freezes, actual muscle contractions
- 5 backs/turns away
- 6 walks past probe
- O other reaction, define in free text

Touch response

- 1 no reaction
- 2 slowly turns to side
- 3 walks away
- 4 freezes
- 5 turns to opposite side
- 6 walks backwards
- O other reaction, define in free text

Startle response

- 1 no reaction
- 2 ear twitch only
- 3 normal flinch response (head and shoulders move)
- 4 noticeable response (whole body movement)
- 5 exaggerated response (all four feet off floor)
- O other reaction, define in free text

Tail pinch

- 1 no reaction
- 2 turns
 - 2 turns immediately
 - 3 violent turn
- 3 walks away
- 4 freezes
- 5 jumps forward
- 6 runs away
- O Other reaction - see additional comments
 - A 1 with a response that is not a turn indicates that the response included a turn such as walks away with a turn

APPENDIX 4**(Functional observational battery - continued)****Righting reflex**

- 1 immediate reaction
- 2 reaction slow

Vocalising, grooming

- Y sign observed
- N sign not observed

the numbers associated with vocalising indicate the degree of effect
1,2,3 increasing loudness of vocalising

Pupil reflex

- B reflex observed both eyes
- L/R reflex observed in left/right eye only
- N no reflex both eyes

Urine

- N none observed
- S small amount observed
- M moderate amount observed
- L large amount observed

Rearing and activity counts

Counts were made of rearing and activity when the animals were in the arena. A count for rearing was counted every time the animal lifted both fore feet clear of a supporting surface. The floor of the arena was marked off into 4 equal areas ("squares"). A count for activity was made whenever the animal moved all four feet into one of these squares.

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 1 males, Control				
Animal no	6	7	8	9	10
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	Y	N	N
degree			1		
IN THE ARENA					
Tremors					
Grooming	N	Y	N	Y	Y
Activity count	12	13	13	17	4
Arousal	4	4	4	4	3
Rearing count	13	9	13	14	4
Bolus count	0	0	0	0	0
Urine present	N	M	S	M	S
Gait					
MANIPULATIONS					
Approach	3	3	5	3	3
Touch	3	4	1	3	3
vocalises					
degree					
Startle	3	4	2	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	6	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	1	1	1	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.3	38.3	38.1	38.0	37.7
Bodyweight (g)	196	188	191	194	186
GRIP STRENGTH (kg)#					
Forelimb	0.90	0.79	0.76	0.70	0.65
Hindlimb	0.64	0.75	0.73	0.84	0.59
FOOTSPLAY (cm) #	8.5	9.6	10.7	9.4	8.3

Values represent the mean of two trials

Additional comments

Animal no.

- 6 Slight brown nasal staining; matted fur lower jaw
- 7 In the arena: whole body grooming
- 8 Slight brown nasal staining
- 9 Slight brown nasal staining; general yellow staining of fur
- 10 Slight brown nasal staining
- In the arena: sitting on edge of arena
- During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 1 males, Control				
Animal no	81	82	83	84	85
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	3	2	2	2
Salivation	Y	N	N	N	N
degree	1				
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	Y	Y	N	N	Y
Activity count	6	7	18	14	3
Arousal	4	4	4	4	4
Rearing count	9	12	8	11	6
Bolus count	4	0	0	0	0
Urine present	S	S	N	N	S
Gait					
MANIPULATIONS					
Approach	3	3	5	3	3
Touch	3	5	1	3	3
vocalises					
degree					
Startle	3	2	2	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	6	3	3
turns			1		
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	1	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	39.1	37.6	37.4	38.2	37.8
Bodyweight (g)	207	197	203	194	206
GRIP STRENGTH (kg) #					
Forelimb	0.69	0.84	0.55	0.64	0.86
Hindlimb	0.76	0.87	0.63	0.66	0.88
FOOTSPREAD (cm) #	7.7	10.9	8.5	5.5	10.0

Values represent the mean of two trials

Additional comments

Animal no.

- 81 In the arena: sitting on edge of arena
- 82 Slight brown nasal staining
- 83 Generalised slight yellow staining of fur
In the arena: initial slipping of all limbs
During manipulations: developed slight salivation
- 84 Generalised slight yellow staining of fur
- 85 Generalised slight yellow staining of fur

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 2 males, 200 ppm				
Animal no	16	17	18	19	20
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	Y	N	N	N	N
degree	1				
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	Y	N	Y	Y	N
Activity count	19	4	10	2	7
Arousal	4	4	4	4	4
Rearing count	11	3	14	6	10
Bolus count	0	3	0	4	2
Urine present	N	S	N	N	S
Gait				U	
MANIPULATIONS					
Approach	3	5	3	3	3
Touch	2	2	2	5	3
vocalises					
degree					
Startle	4	3	3	2	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	5	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	3	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.5	38.4	37.6	37.3	37.3
Bodyweight (g)	201	190	191	188	192
GRIP STRENGTH (kg) #					
Forelimb	0.85	0.77	0.86	0.80	0.76
Hindlimb	0.83	0.76	0.87	0.62	0.67
FOOTSPRAY (cm) #					
	12.8	10.6	7.8	9.1	9.4

Values represent the mean of two trials

Additional comments

Animal no.

- 16 Slight hairloss neck
In the arena: slight slipping of hindlimbs initially
- 17 Slight brown nasal staining
- 18 In the arena: whole body grooming
During manipulations: faeces pale
- 19 In the arena: initial slipping all feet; sitting on edge of arena; eyes partial to half-closed occasionally

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 2 males, 200 ppm				
Animal no	86	87	88	89	90
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors				B1	
Grooming	Y	N	Y	N	Y
Activity count	5	3	8	1	2
Arousal	4	4	4	3	3
Rearing count	2	5	4	5	7
Bolus count	0	0	2	4	0
Urine present	M	N	N	M	N
Gait				U	U
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	0	5	3	4	3
vocalises				Y	
degree				2	
Startle	3	2	4	3	3
Righting reflex	1	2	1	1	1
Tail pinch	3	3	3	3	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	2	1	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.4	38.1	38.8	38.0	37.5
Bodyweight (g)	188	198	195	209	194
GRIP STRENGTH (kg) #					
Forelimb	0.74	0.76	0.56	0.76	0.72
Hindlimb	0.74	0.70	0.61	0.81	0.82
FOOTSPLAY (cm) #	7.9	7.9	6.6	8.7	10.7

Values represent the mean of two trials

Additional comments

Animal no.

- 86 Touch response: runs away
- 87 Slight brown nasal staining
- 88 Generalised slight yellow staining of fur
- In the arena: sitting on edge of arena
- 89 Toenail right forepaw damaged
- In the arena: sitting on edge of arena; slipping of hindlimbs occasionally; red discharge noted (from damaged toenail); sitting in corner
- 90 In the arena: sitting on edge of arena

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 3 males, 1000 ppm				
Animal no	26	27	28	29	30
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	Y	N	Y	N	N
degree	1		1		
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	Y	N	Y	N	N
Activity count	10	10	9	10	10
Arousal	4	4	4	4	4
Rearing count	6	5	8	9	9
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait					
MANIPULATIONS					
Approach	3	2	5	5	5
Touch	1	1	3	1	2
vocalises					
degree					
Startle	3	3	3	4	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	2	2	1	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.9	37.2	38.2	37.7	37.7
Bodyweight (g)	184	200	184	195	188
GRIP STRENGTH (kg) #					
Forelimb	0.81	0.93	0.76	0.69	0.80
Hindlimb	0.64	0.81	0.71	0.46	0.68
FOOTSPRAY (cm) #	9.9	8.6	8.4	7.2	7.7

Values represent the mean of two trials

Additional comments

Animal no.

- 26 In the arena: whole body grooming
During manipulations: faeces pale
- 27 Slight brown nasal staining
- 28 Slight hair loss on neck
- 29 Moderate brown nasal staining
In the arena: initial slipping of all four feet; sitting on edge of arena
- 30 Slight hairloss neck
In the arena: sitting on edge of arena

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 3 males, 1000 ppm				
Animal no	91	92	93	94	95
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	N	N	N
degree		2			
IN THE ARENA					
Tremors					
Grooming	N	N	N	Y	N
Activity count	6	4	11	8	9
Arousal	4	4	4	4	4
Rearing count	9	6	8	10	6
Bolus count	0	0	1	0	1
Urine present	N	N	N	N	N
Gait					
MANIPULATIONS					
Approach	3	5	3	3	5
Touch	3	5	5	1	3
vocalises					
degree					
Startle	3	3	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	1				1
vocalises	Y	Y	Y		Y
degree	2	2	1		2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.6	38.7	37.8	37.6	37.1
Bodyweight (g)	205	203	192	201	193
GRIP STRENGTH (kg) #					
Forelimb	0.70	0.81	0.68	0.75	0.62
Hindlimb	0.95	0.60	0.98	0.75	0.66
FOOTSPLAY (cm) #	7.7	10.2	8.8	9.2	7.6

Values represent the mean of two trials

Additional comments

Animal no.

- 92 In the arena: sitting on edge of arena; head shake
 93 Slight brown nasal staining
 95 Generalised slight yellow staining of fur; patchy hairloss neck

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 4 males, 5000 ppm				
Animal no	36	37	38	39	40
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	Y	N	N
degree			2		
IN THE ARENA					
Tremors					
Grooming	Y	Y	N	N	Y
Activity count	1	7	13	9	9
Arousal	3	4	4	4	4
Rearing count	4	7	15	9	7
Bolus count	1	0	0	0	0
Urine present	S	S	S	N	N
Gait	U				
MANIPULATIONS					
Approach	3	0	3	3	3
Touch	3	1	5	3	3
vocalises					
degree					
Startle	3	3	2	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns		1			
vocalises	Y	Y	Y	Y	Y
degree	2	1	3	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.6	38.3	37.8	38.0	38.0
Bodyweight (g)	198	195	188	183	188
GRIP STRENGTH (kg) #					
Forelimb	0.87	0.82	0.71	0.62	0.64
Hindlimb	0.76	0.78	0.82	0.61	0.81
FOOTSPRAY (cm) #	11.1	11.8	8.4	7.0	11.3

Values represent the mean of two trials

Additional comments

Animal no.

- 36 Slight brown nasal staining; slight brown staining on head
In the arena: sitting in corner
- 37 Generalised slight yellow staining of fur
Approach response: walked past probe
- 38 Matted fur lower jaw
During manipulations: vocalising moderately
Touch response: huffed
- 39 General yellow staining of fur
In the arena: initial slipping of all four feet

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 4 males, 5000 ppm				
Animal no	96	97	98	99	100
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	Y	N	N	N
degree		1			
Vocalising	N	N	N	N	Y
degree					1
IN THE ARENA					
Tremors		B1			
Grooming	N	Y	Y	Y	N
Activity count	7	7	9	9	2
Arousal	4	4	4	4	2
Rearing count	11	9	7	9	0
Bolus count	2	0	0	0	0
Urine present	S	N	N	N	S
Gait	A1				U
MANIPULATIONS					
Approach	3	3	6	3	3
Touch	3	2	5	2	2
vocalises					
degree					
Startle	3	3	3	2	4
Righting reflex	1	1	1	1	1
Tail pinch	3	3	5	3	3
turns				1	
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	38.3	38.1	37.7	38.1
Bodyweight (g)	198	199	209	197	192
GRIP STRENGTH (kg) #					
Forelimb	0.78	0.71	0.82	0.83	0.99
Hindlimb	0.79	0.70	0.87	0.78	0.96
FOOTSPLAY (cm) #	10.2	10.2	6.0	9.2	9.7

Values represent the mean of two trials

Additional comments

Animal no.

- 96 Generalised slight yellow staining of fur
In the arena: sitting on edge of arena
- 97 Slight brown nasal staining; slight hairloss neck
- 98 Slight yellow staining of fur back
In the arena: sitting on edge of arena
- 99 Slight brown nasal staining; generalised slight yellow staining of fur
In the arena: scratching
- 100 Slight brown nasal staining
In the arena: sitting in corner

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 1 females, Control				
Animal no	46	47	48	49	50
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	N	N	N
degree		1			
IN THE ARENA					
Tremors					
Grooming	N	Y	Y	N	N
Activity count	13	1	6	1	9
Arousal	4	4	4	4	4
Rearing count	15	2	6	6	6
Bolus count	0	0	0	0	0
Urine present	N	N	S	S	N
Gait		U		U	
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	1	3	3	1
vocalises					
degree					
Startle	3	3	2	3	3
Righting reflex	1	1	1	1	1
Tail pinch	6	3	6	3	3
turns					1
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	39.3	38.4	38.1	38.0	38.2
Bodyweight (g)	160	156	151	153	151
GRIP STRENGTH (kg) #					
Forelimb	0.67	0.68	0.76	0.62	0.77
Hindlimb	0.75	0.78	0.79	0.54	0.63
FOOTSPRAY (cm) #	7.2	5.9	10.8	5.2	7.1

Values represent the mean of two trials

Additional comments

Animal no.

- 47 Patchy hairloss back
In the arena: sitting on edge of arena
- 49 Lower teeth pale
In the arena: sitting on edge of arena

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 1 females, Control				
Animal no	101	102	103	104	105
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	Y	N	N
Activity count	3	5	7	4	9
Arousal	4	4	4	3	4
Rearing count	6	11	10	4	10
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	U			U	
MANIPULATIONS					
Approach	5	3	3	3	3
Touch	3	3	3	3	3
vocalises					
degree					
Startle	3	3	3	3	3
Righting reflex	2	1	1	1	1
Tail pinch	3	6	3	3	3
turns					
vocalises	Y	Y	Y	Y	
degree	2	2	2	2	
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.3	37.4	37.8	38.4	38.3
Bodyweight (g)	173	173	179	163	162
GRIP STRENGTH (kg) #					
Forelimb	0.80	0.73	0.92	0.56	0.59
Hindlimb	0.88	0.81	0.90	0.79	0.66
FOOTSPRAY (cm) #	7.7	9.5	10.1	6.8	6.1

Values represent the mean of two trials

Additional comments

Animal no.

- 101 In the arena: limited walking
- 104 Lower teeth pale; slight generalised yellow staining of fur
- In the arena: sitting/walking along edge of arena
- 105 In the hand: slight brown nasal staining

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 2 females, 200 ppm				
Animal no	56	57	58	59	60
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	Y	N	Y
Activity count	16	6	6	13	12
Arousal	4	4	4	4	4
Rearing count	14	8	13	9	4
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait					
MANIPULATIONS					
Approach	3	5	5	3	3
Touch	1	1	3	3	2
vocalises					
degree					
Startle	2	3	3	3	4
Righting reflex	1	1	1	1	1
Tail pinch	6	3	3	3	3
turns					
vocalises	Y		Y	Y	Y
degree	2		2	1	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	39.3	37.9	38.2	38.0	38.4
Bodyweight (g)	171	155	165	150	160
GRIP STRENGTH (kg) #					
Forelimb	0.86	0.68	0.48	0.84	0.80
Hindlimb	0.78	0.87	0.60	0.85	0.83
FOOTSPRAY (cm) #					
	10.5	11.2	6.0	8.1	11.0

Values represent the mean of two trials

Additional comments

Animal no.

- 56 Lower teeth pale
 58 Slight brown nasal staining; slight brown staining head;
 lower teeth pale; generalised slight yellow staining of
 fur
 59 In the arena: initial slipping of hindlimbs

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 2 females, 200 ppm				
Animal no	106	107	108	109	110
OBSERVATIONS					
IN THE CAGE					
Posture	R	S	S	R	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	N
degree		1	1		
IN THE ARENA					
Tremors					
Grooming	N	N	N	Y	Y
Activity count	1	16	5	10	9
Arousal	3	4	4	4	4
Rearing count	4	14	9	10	6
Bolus count	0	0	0	0	0
Urine present	N	N	S	N	N
Gait	U				
MANIPULATIONS					
Approach	5	3	3	6	3
Touch	3	2	3	2	3
vocalises					
degree					
Startle	3	3	3	4	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns			1		1
vocalises	Y	Y	Y		Y
degree	2	2	3		2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	37.8	38.1	38.8	38.0
Bodyweight (g)	158	162	173	170	167
GRIP STRENGTH (kg) #					
Forelimb	0.73	0.77	0.83	0.82	0.53
Hindlimb	0.76	0.97	0.84	0.78	0.70
FOOTSPLAY (cm) #	8.5	5.8	7.5	6.2	8.9

Values represent the mean of two trials

Additional comments

Animal no.

- 106 In the arena: sitting in corner
 108 Generalised slight yellow staining of fur
 In the arena: sitting on edge of arena
 110 During manipulations: head shake

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 3 females, 1000 ppm				
Animal no	66	67	68	69	70
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	N	Y	N	N	N
degree		1			
Vocalising	N	N	N	Y	N
degree				1	
IN THE ARENA					
Tremors					
Grooming	Y	N	N	N	N
Activity count	17	13	4	14	3
Arousal	4	4	4	4	3
Rearing count	14	12	2	12	3
Bolus count	0	0	0	0	0
Urine present	N	N	N	S	N
Gait					U
MANIPULATIONS					
Approach	5	5	3	3	3
Touch	3	2	3	3	3
vocalises					
degree					
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	6	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	1	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.4	38.5	38.1	37.9	37.8
Bodyweight (g)	152	166	155	147	156
GRIP STRENGTH (kg) #					
Forelimb	0.67	0.67	0.70	0.76	0.68
Hindlimb	0.59	0.90	0.60	0.74	0.86
FOOTSPLAY (cm) #	5.8	12.1	5.6	9.8	7.8

Values represent the mean of two trials

Additional comments

Animal no.

- 67 Generalised slight yellow staining of fur
- 68 Slight brown nasal staining; slight matted fur on rump
- 69 Generalised slight yellow staining of fur
In the arena: sitting on edge of arena; initial slipping of hindlimbs
- 70 Generalised slight yellow staining of fur
In the arena: limited walking
During manipulations: faeces pale and soft

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 3 females, 1000 ppm				
Animal no	111	112	113	114	115
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	R	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	Y	N	N
degree			2		
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	N
Activity count	6	4	6	7	12
Arousal	4	4	3	4	4
Rearing count	8	7	3	5	10
Bolus count	0	0	0	0	0
Urine present	N	S	S	N	N
Gait				U	
MANIPULATIONS					
Approach	3	5	3	3	3
Touch	3	5	3	3	3
vocalises					
degree					
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	2
turns					2
vocalises	Y	Y	Y	Y	Y
degree	2	2	3	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	38.1	38.0	39.3	38.5
Bodyweight (g)	181	166	164	161	160
GRIP STRENGTH (kg) #					
Forelimb	0.85	0.95	0.98	0.74	0.66
Hindlimb	0.96	0.64	0.77	0.80	1.02
FOOTSPLAY (cm) #	10.5	11.5	11.0	4.4	6.1

Values represent the mean of two trials

Additional comments

Animal no.

- 112 In the arena: sitting on edge of arena
- 113 Slight brown nasal staining
- During manipulations: developed red nasal discharge
- 114 Generalised slight yellow staining of fur
- In the arena: sitting / walking on edge of arena
- 115 In the arena: sitting on edge of arena; tail curling occasionally

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 4 females, 5000 ppm				
Animal no	76	77	78	79	80
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	Y
degree					1
IN THE ARENA					
Tremors					
Grooming	N	Y	N	N	Y
Activity count	9	12	9	6	12
Arousal	4	4	4	4	4
Rearing count	11	17	11	5	14
Bolus count	0	0	0	2	0
Urine present	N	N	N	L	N
Gait		T1			
MANIPULATIONS					
Approach	3	3	3	3	6
Touch	2	3	3	2	3
vocalises					
degree					
Startle	3	2	3	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	6	3
turns		1			
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	38.2	37.5	38.2	37.9
Bodyweight (g)	163	171	160	162	161
GRIP STRENGTH (kg) #					
Forelimb	0.73	0.78	0.66	0.84	0.70
Hindlimb	0.77	0.61	0.68	0.95	0.81
FOOTSPRAY (cm) #	9.4	6.2	5.8	11.1	9.0

Values represent the mean of two trials

Additional comments

Animal no.

- 76 Slight brown nasal staining
- 77 Lower teeth pale; generalised slight yellow staining of fur
- 78 Lower teeth pale; generalised slight yellow staining of fur
In the arena: sitting on edge of arena; head shake
- 79 Slight hairloss neck
During temperature: slight body tremors
- 80 In the arena: head shake
During manipulations: vocalising moderately

APPENDIX 4

(Functional observational battery - continued)

Pre-dose	Group 4 females, 5000 ppm				
Animal no	116	117	118	119	120
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	N	N	N
degree		2			
IN THE ARENA					
Tremors					
Grooming	N	Y	N	N	Y
Activity count	10	10	5	10	3
Arousal	4	4	4	4	3
Rearing count	9	10	5	9	3
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait					U
MANIPULATIONS					
Approach	5	3	3	3	3
Touch	2	3	2	3	3
vocalises					
degree					
Startle	4	3	3	3	3
Righting reflex	1	1	1	1	2
Tail pinch	3	3	3	3	3
turns	1			1	
vocalises	Y	Y	Y	Y	Y
degree	2	2	1	1	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	37.8	37.6	38.0	38.7
Bodyweight (g)	159	166	181	179	173
GRIP STRENGTH (kg) #					
Forelimb	0.67	0.68	0.80	0.65	0.65
Hindlimb	0.46	0.87	0.69	0.85	0.57
FOOTSPLAY (cm) #					
	6.2	9.2	10.5	7.7	8.4

Values represent the mean of two trials

Additional comments

Animal no.

- 117 During manipulations: vocalising moderately
- 118 In the arena: sitting on edge of arena
- 119 Generalised slight yellow staining of fur
- 120 Lower teeth pale
- In the arena: sitting on edge of arena

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 1 males, Control				
Animal no	6	7	8	9	10
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors	B1	B1			B1
Grooming	N	N	N	N	N
Activity count	6	1	3	9	3
Arousal	4	3	4	4	3
Rearing count	6	3	9	13	0
Bolus count	0	0	0	0	0
Urine present	N	S	N	S	N
Gait		U			U
MANIPULATIONS					
Approach	3	3	5	3	5
Touch	3	3	1	1	4
Startle	3	3	3	4	3
Righting reflex	1	1	1	1	1
Tail pinch	3	2	3	5	2
turns		2		1	3
vocalises		Y	Y	Y	Y
degree		2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	38.2	37.7	37.3	38.4
Bodyweight (g)	379	357	387	395	369
GRIP STRENGTH (kg) #					
Forelimb	1.69	1.03	1.56	1.32	0.86
Hindlimb	1.19	1.02	1.31	1.02	1.12
FOOTSPRAY (cm) #					
	10.2	7.2	12.1	6.5	11.0

Values represent the mean of two trials

Additional comments

Animal no.

- 6 Slight lack of grooming back; moderate brown nasal staining
During temperature: slightly body tremors
- 7 Slight lack of grooming back; slight brown nasal staining
In the arena: sitting in corner
- 8 Slight lack of grooming back; slight brown nasal staining
In the arena: head shake
- 9 Slight lack of grooming back; slight brown nasal staining
- 10 Slightly hairloss rump; slight brown nasal staining; slight
lack of grooming rump
In the arena: head slightly tilted to the right; limited
walking
During temperature: moderate body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 1 males, Control					
	Animal no	81	82	83	84	85
OBSERVATIONS						
IN THE CAGE						
Posture		S	R	S	R	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		Y	N	Y	N	N
degree		1		1		
Vocalising		N	N	N	N	N
degree						
IN THE ARENA						
Tremors						
Grooming		Y	N	N	N	N
Activity count		10	4	5	12	9
Arousal		4	4	4	4	4
Rearing count		15	6	9	16	8
Bolus count		0	0	0	0	0
Urine present		M	N	N	N	S
Gait						
MANIPULATIONS						
Approach		3	3	3	3	5
Touch		3	4	1	3	1
Startle		3	3	2	3	2
Righting reflex		1	1	1	1	1
Tail pinch		3	3	6	3	6
turns		1			1	
vocalises		Y	Y	Y	Y	Y
degree		2	2	2	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		38.7	38.1	37.7	37.9	37.3
Bodyweight (g)		403	333	376	364	366
GRIP STRENGTH (kg) #						
Forelimb		1.38	1.59	1.25	0.98	1.55
Hindlimb		1.24	1.31	1.16	1.12	1.35
FOOTSPRAY (cm) #						
		9.7	10.3	10.1	6.1	9.1

Values represent the mean of two trials

Additional comments

Animal no.

- 81 Slight lack of grooming; slightly emaciated
- 82 Slight brown nasal staining
- During temperature: slight body tremors
- 83 Slight brown nasal staining; slight lack of grooming back
- 84 Slight lack of grooming back
- 85 Slight lack of grooming back
- During temperature: head shake

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 2 males, 200 ppm					
	Animal no	16	17	18	19	20
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	R	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	3	2	2	2
Salivation		Y	N	N	N	N
degree		1				
Vocalising		N	Y	Y	Y	N
degree			2	1	1	
IN THE ARENA						
Tremors						
Grooming		N	N	N	N	N
Activity count		2	11	19	2	1
Arousal		3	4	4	3	3
Rearing count		3	11	20	6	2
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait		U			U	U
MANIPULATIONS						
Approach		3	3	3	3	3
Touch		5	1	3	1	3
Startle		3	3	3	4	2
Righting reflex		1	1	1	1	1
Tail pinch		2	6	6	6	3
turns		2	1			
vocalises		Y	Y	Y	Y	Y
degree		2	2	3	3	1
Pupil reflex		B	B	B	L	B
Temperature (°C)		39.1	39.1	38.5	37.3	37.5
Bodyweight (g)		391	395	344	354	397
GRIP STRENGTH (kg) #						
Forelimb		1.12	1.54	1.37	1.33	1.37
Hindlimb		1.49	1.33	1.28	1.20	1.10
FOOTSPRAY (cm) #						
		12.3	11.3	9.7	9.1	9.8

Values represent the mean of two trials

Additional comments

Animal no.

- 16 Slight lack of grooming back; all feet cold to touch
(appearance normal)
In the arena: sitting in corner
During manipulations: red discharge from inner digit right hindpaw
- 17 Slight lack of grooming back; slight brown nasal staining
- 18 Slight lack of grooming back
- 19 Slight lack of grooming back; slightly emaciated
In the arena: sitting in corner
Pupil reflex: right pupil constricted
- 20 Slight lack of grooming back
In the arena: sitting in corner
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 2 males, 200 ppm				
Animal no	86	87	88	89	90
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	N	N	N	Y
degree	2				2
IN THE ARENA					
Tremors					B1
Grooming	N	N	Y	N	Y
Activity count	16	2	9	1	4
Arousal	4	3	4	3	4
Rearing count	8	4	10	2	4
Bolus count	0	0	1	1	0
Urine present	N	N	N	S	N
Gait		U	T1	U	
MANIPULATIONS					
Approach	3	5	3	3	3
Touch	4	1	3	4	1
Startle	4	3	3	2	3
Righting reflex	1	2	2	1	1
Tail pinch	3	3	3	3	6
turns					
vocalises	Y	Y	Y	Y	Y
degree	3	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.4	37.8	38.5	38.0	37.7
Bodyweight (g)	334	399	358	365	390
GRIP STRENGTH (kg) #					
Forelimb	1.64	1.33	1.03	1.21	1.34
Hindlimb	1.29	1.14	0.97	1.22	1.14
FOOTSPRAY (cm) #	9.5	8.3	9.3	6.6	9.5

Values represent the mean of two trials

Additional comments

Animal no.

- 86 During grip strength: vocalising moderately
- 87 Lack of grooming back; slight brown nasal staining
In the arena: limited walking
- 88 Slight lack of grooming back
In the arena: whole body grooming
- 89 Claw missing on digit left forepaw and digit red in colour;
slight lack of grooming back
In the arena: sitting in corner
- 90 Slight brown nasal staining; slight lack of grooming back
In the arena: tail curling occasionally
During manipulations: developed slight salivation
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4		Group 3 males, 1000 ppm				
	Animal no	26	27	28	29	30
OBSERVATIONS						
IN THE CAGE						
Posture		S	R	S	S	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	Y	N	N
degree				1		
Vocalising		N	Y	N	N	Y
degree			2			2
IN THE ARENA						
Tremors		B1				B1
Grooming		Y	N	Y	N	Y
Activity count		8	3	7	8	3
Arousal		4	3	4	4	4
Rearing count		7	4	6	7	4
Bolus count		0	0	0	0	0
Urine present		S	N	N	S	N
Gait			U		T1	U
MANIPULATIONS						
Approach		5	3	3	3	3
Touch		3	1	4	3	4
Startle		3	2	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		5	3	6	3	6
turns					1	1
vocalises			Y	Y	Y	Y
degree			2	3	2	3
Pupil reflex		B	B	B	B	B
Temperature (°C)		38.2	38.6	38.7	37.4	37.2
Bodyweight (g)		363	374	381	350	364
GRIP STRENGTH (kg) #						
Forelimb		1.13	1.34	1.26	1.26	1.10
Hindlimb		0.91	1.25	1.29	1.14	1.02
FOOTSPRAY (cm) #						
		5.5	13.1	10.2	9.5	8.5

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 26 Slight lack of grooming back; slight brown nasal staining;
all feet cold to touch (appearance normal); moderate
hairloss both shoulders; slight scabs left shoulder
During manipulations: developed slight salivation
- 27 Slight brown nasal staining; slight lack of grooming back;
slight kink tip of tail
In the arena: limited walking; faeces soft
Righting reflex: slow to complete
Holding for tail pinch: position stationary
During grip strength: vocalising moderately
- 28 Moderate lack of grooming back
In the arena: eyes partially to half-closed occasionally
- 29 Slight lack of grooming back; slight brown nasal staining
During temperature: slight body tremors
- 30 Slight lack of grooming back
In the arena: eyes partially to half-closed occasionally;
limited walking
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 3 males, 1000 ppm					
	Animal no	91	92	93	94	95
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	S	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	Y	N	N	N
degree			2			
IN THE ARENA						
Tremors						
Grooming		N	N	N	N	N
Activity count		12	7	11	5	11
Arousal		4	4	4	4	4
Rearing count		8	12	9	8	5
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait						
MANIPULATIONS						
Approach		2	3	3	2	3
Touch		1	4	4	1	3
Startle		2	3	4	2	2
Righting reflex		1	1	1	1	1
Tail pinch		3	3	5	3	3
turns		1	1			1
vocalises		Y	Y	Y	Y	Y
degree		2	2	1	1	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		38.5	38.4	37.7	37.7	37.4
Bodyweight (g)		363	378	349	388	380
GRIP STRENGTH (kg) #						
Forelimb		1.11	1.25	1.21	1.40	1.16
Hindlimb		1.42	0.90	1.29	1.47	0.96
FOOTSPRAY (cm) #						
		7.3	10.7	9.1	9.5	8.7

Values represent the mean of two trials

Additional comments

Animal no.

- 91 Moderate lack of grooming; slight brown nasal staining
- 92 Slight lack of grooming back
During temperature: slight body tremors
- 93 Slight lack of grooming back
- 94 Slight lack of grooming back
During temperature: head shake five times
- 95 Slight lack of grooming back
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 4 males, 5000 ppm				
Animal no	36	37	38	39	40
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	N	Y	N	Y
degree	1		3		1
IN THE ARENA					
Tremors					
Grooming	N	Y	N	N	Y
Activity count	5	15	3	2	8
Arousal	4	4	3	4	4
Rearing count	6	15	3	5	6
Bolus count	1	0	0	0	0
Urine present	N	N	S	N	N
Gait		T1	T2		
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	1	4	0	2	3
Startle	3	3	3	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	6	2	6
turns				2	
vocalises	Y	Y	Y		Y
degree	2	1	3		2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.6	37.9	38.5	38.9	37.9
Bodyweight (g)	339	295	307	318	353
GRIP STRENGTH (kg) #					
Forelimb	1.15	0.84	1.10	0.74	0.92
Hindlimb	0.93	0.68	0.89	0.67	0.66
FOOTSPRAY (cm) #	12.9	8.7	7.2	6.4	9.8

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 36 Slight lack of grooming back
- 37 Slight lack of grooming back; slight brown nasal staining;
slightly emaciated
In the arena: initially rock/sway movements
During manipulations: developed slight salivation and slight
red nasal discharge
Righting reflex: slow to complete
During temperature: slight body tremors
- 38 Slight lack of grooming back; slightly emaciated
Touch response: whole body muscle contractions (appears to
be retching)
During manipulations: faeces soft
Startle response: vocalising moderately
- 39 Moderate lack of grooming back; slightly emaciated
- 40 Slight lack of grooming back; abdomen slightly firm
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 4 males, 5000 ppm				
Animal no	96	97	98	99	100
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors			B1		B1
Grooming	N	Y	Y	Y	N
Activity count	1	4	5	12	3
Arousal	3	3	4	4	3
Rearing count	1	7	10	10	4
Bolus count	2	0	0	0	0
Urine present	M	S	S	N	S
Gait	U				U
MANIPULATIONS					
Approach	3	3	3	3	2
Touch	3	1	3	4	1
Startle	3	3	3	2	4
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	2	6
turns				2	
vocalises	Y		Y	Y	Y
degree	2		2	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	38.7	37.9	37.1	37.5
Bodyweight (g)	360	320	359	348	323
GRIP STRENGTH (kg) #					
Forelimb	1.34	0.90	1.17	1.25	1.62
Hindlimb	1.36	0.72	1.10	1.20	1.22
FOOTSPLAY (cm) #	11.8	10.7	7.0	10.4	10.0

Values represent the mean of two trials

Additional comments

Animal no.

- 96 Slight lack of grooming back
In the arena: sat in corner
During temperature: moderate body tremors
- 97 Slight hairloss forefeet; slight red staining right eye;
slight brown nasal staining; slightly emaciated
In the cage: rapid breathing
- 98 Slight brown nasal staining; slight lack of grooming back
In the arena: eyes partially to half-closed occasionally
- 99 Slight brown nasal staining; slight brown staining head;
slight lack of grooming back
During temperature: slight body tremors
- 100 Slight lack of grooming back; slightly emaciated
In the arena: limited walking

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 1 females, Control				
Animal no	46	47	48	49	50
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	N	N	N
degree		1			
IN THE ARENA					
Tremors					
Grooming	N	N	Y	N	N
Activity count	23	15	13	2	12
Arousal	4	4	4	4	4
Rearing count	15	16	15	5	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T1		U	
MANIPULATIONS					
Approach	5	3	3	0	3
Touch	2	3	4	3	3
Startle	3	4	4	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns		1			
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	39.5	38.9	38.4	37.6	38.7
Bodyweight (g)	232	197	208	210	193
GRIP STRENGTH (kg) #					
Forelimb	0.83	0.93	1.26	0.74	0.95
Hindlimb	1.07	1.23	1.20	0.87	0.78
FOOTSPLAY (cm) #					
	5.5	5.5	11.4	5.1	6.7

Values represent the mean of two trials

Additional comments

Animal no.

- 46 Slight lack of grooming rump
- 47 During manipulations: slightly awkward to handle and vocalising moderately
- 49 In the arena: limited walking/sitting in corner
Approach response: walked away
- 50 Slight brown staining neck; slight lack of grooming back

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 1 females, Control					
	Animal no	101	102	103	104	105
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	R	S	R
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	Y	N	N
degree				2		
IN THE ARENA						
Tremors						
Grooming		Y	Y	Y	N	N
Activity count		22	5	14	4	24
Arousal		4	4	4	4	4
Rearing count		17	10	9	1	18
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait		T2			U	
MANIPULATIONS						
Approach		3	5	3	3	6
Touch		3	5	3	3	3
Startle		3	2	4	3	3
Righting reflex		1	1	1	1	1
Tail pinch		5	3	3	3	3
turns						
vocalises		Y	Y	Y	Y	
degree		2	2	2	2	
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.4	37.0	38.4	38.5	38.5
Bodyweight (g)		247	248	265	227	233
GRIP STRENGTH (kg) #						
Forelimb		1.21	1.15	1.42	0.88	0.83
Hindlimb		1.11	1.26	1.48	0.98	1.33
FOOTSPRAY (cm) #						
		6.1	8.9	11.9	9.7	7.6

Values represent the mean of two trials

Additional comments

Animal no.

- 101 Slight brown nasal staining; slight lack of grooming back
- 103 Slight brown nasal staining; slight brown staining head
- 104 Slight brown nasal staining
- In the arena: sitting on/walking along edge of arena
- 105 Moderate hairloss forelimbs; slight lack of grooming back

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 2 females, 200 ppm				
Animal no	56	57	58	59	60
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	Y
Activity count	3	13	15	12	19
Arousal	4	4	4	4	4
Rearing count	5	15	15	7	19
Bolus count	0	0	0	0	0
Urine present	N	S	N	N	N
Gait					
MANIPULATIONS					
Approach	5	3	3	3	3
Touch	3	3	5	5	5
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	6	3	3	3	3
turns	1	1			
vocalises	Y	Y	Y	Y	Y
degree	2	1	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	39.3	38.5	39.0	38.3	38.7
Bodyweight (g)	262	224	245	211	231
GRIP STRENGTH (kg) #					
Forelimb	1.33	1.01	0.72	0.97	1.34
Hindlimb	1.40	1.13	0.74	1.29	1.34
FOOTSPRAY (cm) #					
	8.1	6.9	6.5	7.2	7.1

Values represent the mean of two trials

Additional comments

Animal no.

- 56 Slight brown nasal staining; patchy hairloss rump
- 57 Slight lack of grooming rump
- 58 Lower teeth pale
- In the arena: incomplete scratching movement
- 59 Slightly emaciated; slight lack of grooming back
- During temperature: slightly body tremors
- 60 During temperature: vocalising moderately

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 2 females, 200 ppm				
Animal no	106	107	108	109	110
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	R	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	N
degree		1	1		
IN THE ARENA					
Tremors					
Grooming	N	N	N	Y	N
Activity count	7	19	16	18	13
Arousal	4	4	4	4	4
Rearing count	5	10	17	13	10
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait			T1		
MANIPULATIONS					
Approach	3	3	5	3	3
Touch	1	5	1	3	2
Startle	3	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	2	2	5	3	3
turns	2	3	1		
vocalises	Y	Y	Y	Y	
degree	2	2	2	2	
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.7	38.3	38.1	38.3	38.9
Bodyweight (g)	207	236	249	247	241
GRIP STRENGTH (kg) #					
Forelimb	1.04	0.97	1.24	1.33	0.95
Hindlimb	0.80	1.35	1.23	1.01	1.04
FOOTSPRAY (cm) #					
	6.3	8.9	6.2	8.5	8.9

Values represent the mean of two trials

Additional comments

Animal no.

- 106 Slight lack of grooming rump
In the arena: walking backwards
- 107 In the arena: scratching
- 109 Slight lack of grooming back; slightly emaciated
- 110 Slight brown nasal staining

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 3 females, 1000 ppm					
	Animal no	66	67	68	69	70
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	R	R
IN THE HAND						
Removing		3	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	N	Y	N
degree					2	
IN THE ARENA						
Tremors						
Grooming		Y	N	N	Y	N
Activity count		18	19	14	10	6
Arousal		4	4	4	4	4
Rearing count		12	23	18	10	5
Bolus count		0	0	0	0	0
Urine present		M	N	N	N	N
Gait		T2	T1	T1		
MANIPULATIONS						
Approach		5	3	3	3	3
Touch		1	2	3	5	4
Startle		2	3	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		3	3	3	6	3
turns			1			1
vocalises		Y	Y	Y	Y	Y
degree		2	2	2	3	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		38.7	38.9	38.9	37.9	37.5
Bodyweight (g)		205	245	191	211	232
GRIP STRENGTH (kg) #						
Forelimb		1.17	1.12	0.75	1.33	1.00
Hindlimb		0.36	1.20	0.72	1.19	1.07
FOOTSPRAY (cm) #						
		7.3	11.1	5.2	11.3	9.4

Values represent the mean of two trials

Additional comments

Animal no.

- 66 Slight brown nasal staining
- 67 Slight brown nasal staining
- 68 Slightly emaciated; slight brown staining across shoulders
During temperature: vocalising moderately
- 69 Moderate scabs left corner of mouth
In the hand: became slightly awkward to handle
During manipulations: vocalising moderately and moderately
awkward to handle
- 70 Slight brown nasal staining; slight lack of grooming rump

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 3 females, 1000 ppm				
Animal no	111	112	113	114	115
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	Y	N	N
degree			1		
Vocalising	N	Y	Y	N	N
degree		1	1		
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	N
Activity count	5	9	7	23	9
Arousal	4	4	4	4	4
Rearing count	11	7	7	16	10
Bolus count	0	0	0	0	0
Urine present	N	S	N	N	N
Gait				T1	
MANIPULATIONS					
Approach	6	3	3	3	3
Touch	3	3	1	3	3
Startle	2	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	6	3	2
turns		1		1	2
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.4	37.6	38.2	39.2	37.7
Bodyweight (g)	257	219	256	195	209
GRIP STRENGTH (kg) #					
Forelimb	1.33	1.16	1.38	1.14	0.83
Hindlimb	1.34	1.13	1.07	1.08	1.10
FOOTSPLAY (cm) #	11.7	10.0	10.3	6.9	7.7

Values represent the mean of two trials

Additional comments

Animal no.

- 112 Moderate lack of grooming back and rump; slight lack of grooming urogenital region; slightly emaciated
During temperature: slight body tremors
- 113 Slight brown staining head; slight brown staining neck; slight lack of grooming rump
During grip strength and foot splay: vocalising moderately
- 115 Slight lack of grooming back
In the arena: walking along edge of arena

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 4 females, 5000 ppm				
Animal no	76	77	78	79	80
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	R	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	Y
degree					1
IN THE ARENA					
Tremors					
Grooming	N	Y	N	N	Y
Activity count	15	13	14	10	13
Arousal	4	4	4	4	4
Rearing count	14	17	15	14	13
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2HU1	T2HU1			T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	3	3	3	3
Startle	3	2	3	3	3
Righting reflex	2	1	1	1	1
Tail pinch	3	3	3	6	3
turns					
vocalises	Y	Y	Y	Y	Y
degree	1	1	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.8	38.6	38.3	38.5	38.6
Bodyweight (g)	190	222	209	187	190
GRIP STRENGTH (kg) #					
Forelimb	0.42	0.96	0.95	0.70	0.74
Hindlimb	0.30	0.60	0.55	0.34	0.53
FOOTSPRAY (cm) #					
	5.0	5.0	6.6	5.3	5.5

Values represent the mean of two trials

Additional comments

Animal no.

- 76 Slight brown nasal staining; slight brown staining head; slight lack of grooming back; slightly emaciated
- 77 Lower teeth white in colour; slight lack of grooming back; slightly emaciated around hindquarters
- 78 Moderate brown staining both ears; slight lack of grooming rump
- 79 Slightly emaciated
During temperature: slight body tremors
- 80 Slightly emaciated
In the arena: slightly hunched initially

APPENDIX 4

(Functional observational battery - continued)

Week 4	Group 4 females, 5000 ppm				
Animal no	116	117	118	119	120
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	S	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	N	Y	N
Activity count	10	20	5	13	10
Arousal	4	4	4	4	4
Rearing count	12	16	8	14	11
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait		T2		T1	
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	2	3	3	1
Startle	3	1	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns				1	1
vocalises	Y	Y	Y	Y	Y
degree	1	2	2	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.4	39.0	38.4	37.3	38.7
Bodyweight (g)	188	192	239	214	213
GRIP STRENGTH (kg) #					
Forelimb	0.89	0.67	0.98	0.87	0.80
Hindlimb	0.81	0.66	0.58	0.77	0.57
FOOTSPRAY (cm) #	5.5	8.1	9.1	6.4	7.7

Values represent the mean of two trials

Additional comments

Animal no.

- 116 In the arena: sitting on edge of arena
- 117 Slightly emaciated
In the hand: slightly hunched
During foot splay: on first two occasions landed with right hindfoot upside down
- 118 Slight lack of grooming back
In the arena: sitting on edge of arena
- 119 Slightly emaciated; slight lack of grooming back
In the arena: tail curling occasionally
- 120 Slightly emaciated; slight lack of grooming back
In the hand: head shake
In the arena: tail curling occasionally

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 1 males, Control				
Animal no	6	7	8	9	10
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	2
Salivation	Y	N	N	N	N
degree	1				
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	N
Activity count	6	4	5	14	2
Arousal	4	4	4	4	3
Rearing count	7	5	4	16	3
Bolus count	0	0	0	0	0
Urine present	S	S	S	N	S
Gait					U
MANIPULATIONS					
Approach	2	2	5	2	3
Touch	1	1	1	1	4
vocalises					
degree					
Startle	2	3	3	2	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	2
turns		1		1	2
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	1	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.7	38.2	37.6	37.7	38.6
Bodyweight (g)	488	465	496	506	473
GRIP STRENGTH (kg) #					
Forelimb	1.63	1.34	1.66	1.06	1.40
Hindlimb	0.93	1.31	1.28	1.05	1.21
FOOTSPREAD (cm) #					
	10.6	8.0	10.7	7.5	8.5

Values represent the mean of two trials

Additional comments

Animal no.

- 6 Slight brown nasal staining; slight lack of grooming back
- 7 In the arena: eyes partially to half-closed occasionally
- 8 In the arena: eyes partially closed occasionally
During temperature: slight body tremors
- 9 Slight brown nasal staining; slight lack of grooming back
- 10 Slight lack of grooming back
In the arena: limited walking

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 1 males, Control					
	Animal no	81	82	83	84	85
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	R	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	N	N	Y
degree						2
IN THE ARENA						
Tremors						
Grooming		N	N	Y	N	N
Activity count		9	1	12	15	4
Arousal		4	2	4	4	4
Rearing count		9	2	9	10	4
Bolus count		0	1	0	0	0
Urine present		N	N	S	N	S
Gait			U			
MANIPULATIONS						
Approach		3	2	2	3	3
Touch		3	4	1	3	1
vocalises						
degree						
Startle		2	3	2	2	3
Righting reflex		1	1	1	1	1
Tail pinch		3	6	3	3	3
turns				1		
vocalises		Y	Y	Y		Y
degree		2	2	1		2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.7	38.2	38.1	38.4	37.6
Bodyweight (g)		508	424	455	453	450
GRIP STRENGTH (kg) #						
Forelimb		1.30	1.61	1.02	1.22	1.84
Hindlimb		1.09	1.51	0.91	1.32	1.53
FOOTSPLAY (cm) #						
		7.0	10.4	12.3	7.0	11.7

Values represent the mean of two trials

Additional comments

Animal no.

- 81 Slight lack of grooming back; slight brown nasal staining
- 82 Slight lack of grooming back
In the arena: sitting in corner
- 83 Slight lack of grooming back; top teeth crooked; slight brown staining head
- 84 Moderate lack of grooming back; slight brown nasal staining; tip of tail scaly
Following pupil reflex: head shake
- 85 Slight brown nasal staining; slight lack of grooming back; damaged digit left forepaw

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 2 males, 200 ppm				
Animal no	16	17	18	19	20
OBSERVATIONS					
IN THE CAGE					
Posture	R	S	S	R	S
IN THE HAND					
Removing	2	5	2	2	2
Handling	2	5	2	2	2
Salivation	Y	Y	N	N	N
degree	1	1			
Vocalising	N	Y	N	N	N
degree		2			
IN THE ARENA					
Tremors					
Grooming	Y	N	N	N	Y
Activity count	16	15	13	5	17
Arousal	4	4	4	4	4
Rearing count	13	10	13	6	16
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait					T1
MANIPULATIONS					
Approach	5	3	3	5	3
Touch	4	4	4	4	3
vocalises					
degree					
Startle	3	3	4	2	3
Righting reflex	1	1	1	2	1
Tail pinch	2	2	5	5	3
turns	3	2			
vocalises	Y	Y	Y	Y	Y
degree	3	3	3	3	3
Pupil reflex	B	R	B	B	B
Temperature (°C)	37.7	39.1	38.5	36.9	38.1
Bodyweight (g)	490	522	410	452	509
GRIP STRENGTH (kg) #					
Forelimb	1.39	1.77	1.63	1.64	1.68
Hindlimb	1.46	1.35	1.47	1.00	1.44
FOOTSPLAY (cm) #					
	8.4	12.1	8.7	8.3	11.1

Values represent the mean of two trials

Additional comments

Animal no.

- 16 Slight brown nasal staining; slight lack of grooming back
Righting reflex: slow to complete
- 17 Slight lack of grooming back
Pupil reflex: left pupil constricted
During manipulations :moderately awkward to handle and
vocalising moderately
- 18 Slight lack of grooming back
- 19 Slight brown nasal staining; slight brown staining head;
slight hairloss right forelimb; slight lack of grooming
back
- 20 Slight lack of grooming back
Following pupil reflex: head shake

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 2 males, 200 ppm				
	Animal no	86	87	88	89 90
OBSERVATIONS					
IN THE CAGE					
Posture		S	R	S	S R
IN THE HAND					
Removing		2	2	2	2 2
Handling		2	2	2	2 2
Salivation		N	N	N	N N
degree					
Vocalising		N	N	N	N Y
degree					2
IN THE ARENA					
Tremors				B1	
Grooming		Y	N	Y	N N
Activity count		12	4	1	1 4
Arousal		4	4	2	2 3
Rearing count		12	6	2	1 4
Bolus count		0	0	0	0 0
Urine present		S	S	N	S N
Gait				U	U
MANIPULATIONS					
Approach		3	3	2	1 5
Touch		4	3	3	1 4
vocalises					
degree					
Startle		4	3	3	2 2
Righting reflex		1	2	1	1 1
Tail pinch		0	2	5	3 3
turns			2		
vocalises			Y	Y	Y Y
degree			2	2	2 2
Pupil reflex		B	B	B	B B
Temperature (°C)		37.9	38.3	37.8	37.7 37.7
Bodyweight (g)		387	502	471	484 493
GRIP STRENGTH (kg) #					
Forelimb		1.69	1.40	1.53	1.46 1.45
Hindlimb		1.33	1.26	1.41	1.44 0.97
FOOTSPLAY (cm) #		10.5	8.7	10.5	7.6 9.5

Values represent the mean of two trials

Additional comments

Animal no.

- 86 Slight lack of grooming back
During manipulations: vocalising moderately
Tail pinch: jumped with turn pivoting with forelimbs
- 87 Slight lack of grooming back
- 88 Slight lack of grooming back
In the arena: eyes partially closed occasionally
- 89 Slight lack of grooming back
In the arena: sitting in corner
- 90 Slight lack of grooming back; moderate brown nasal staining
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 3 males, 1000 ppm				
Animal no	26	27	28	29	30
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	S	R	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	3	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	Y
degree		1	2		2
IN THE ARENA					
Tremors		B2	B1		
Grooming	N	Y	Y	N	N
Activity count	13	4	9	11	10
Arousal	4	3	4	4	4
Rearing count	10	5	11	12	14
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait		U			Alt1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	5	0	4	5	1
vocalises					
degree					
Startle	3	3	3	2	3
Righting reflex	1	2	1	1	1
Tail pinch	3	3	3	3	3
turns	1				
vocalises	Y	Y	Y	Y	Y
degree	1	2	1	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.8	38.4	37.9	38.1	37.6
Bodyweight (g)	463	498	464	444	449
GRIP STRENGTH (kg) #					
Forelimb	1.43	1.34	1.52	1.65	1.20
Hindlimb	0.98	1.03	1.17	1.14	0.96
FOOTSPLAY (cm) #	6.4	11.8	9.6	9.4	9.8

Values represent the mean of two trials

Additional comments

Animal no.

- 26 Slight lack of grooming back; slight brown nasal staining
- 27 Moderate lack of grooming back
In the arena: limited walking
Touch response: appeared to be retching
- 28 Slight brown nasal staining; slight lack of grooming back
- 29 Slight brown staining head; slight lack of grooming back
- 30 Slight lack of grooming back

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 3 males, 1000 ppm				
Animal no	91	92	93	94	95
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	N
degree		2	1		
IN THE ARENA					
Tremors		B1		B1	B1
Grooming	N	N	N	N	N
Activity count	3	4	11	1	2
Arousal	3	4	4	2	2
Rearing count	4	4	8	1	2
Bolus count	0	0	0	0	0
Urine present	S	N	N	S	S
Gait	U	A1HU1		U	U
MANIPULATIONS					
Approach	5	2	2	1	5
Touch	1	3	1	1	3
vocalises					
degree					
Startle	2	4	3	2	2
Righting reflex	1	1	1	1	1
Tail pinch	2	3	3	3	5
turns	2				
vocalises	Y	Y	Y		Y
degree	3	2	1		2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.3	37.9	37.1	37.1	37.0
Bodyweight (g)	439	478	470	500	488
GRIP STRENGTH (kg) #					
Forelimb	1.29	1.02	1.55	1.48	1.26
Hindlimb	1.64	1.06	1.68	1.42	1.09
FOOTSPLAY (cm) #	9.3	7.8	10.4	8.1	8.2

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 91 Slight lack of grooming back; slight brown nasal staining
In the arena: limited walking
- 92 Moderate lack of grooming back; slight brown nasal staining;
abdomen appears slightly firm
- 93 Moderate hairloss forepaws; slight lack of grooming back
- 94 Slight brown nasal staining; slight lack of grooming back
In the arena: eyes partially to half-closed occasionally;
sitting in corner
During manipulations: head shake
During temperature: slight body tremors
- 95 Slight lack of grooming back; slight scab left forelimb
In the arena: eyes partially to half-closed occasionally;
sitting in corner

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 4 males, 5000 ppm				
Animal no	36	37	38	39	40
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	Y	Y	Y	Y
degree	2	1	3	2	2
IN THE ARENA					
Tremors					
Grooming	Y	N	N	N	Y
Activity count	5	20	2	10	4
Arousal	4	4	3	4	4
Rearing count	8	11	3	11	5
Bolus count	1	0	0	0	0
Urine present	N	S	N	N	S
Gait		T1	U		
MANIPULATIONS					
Approach	1	3	5	3	2
Touch	4	1	0	3	3
vocalises					
degree					
Startle	3	3	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns		1	1	1	
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.9	37.9	38.1	38.6	38.4
Bodyweight (g)	435	384	391	417	473
GRIP STRENGTH (kg) #					
Forelimb	1.67	1.62	1.45	1.18	1.43
Hindlimb	1.43	1.29	1.31	1.44	1.18
FOOTSPRAY (cm) #	13.3	9.8	12.4	8.6	13.8

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 36 Moderate lack of grooming back
During manipulations: vocalising moderately
Touch response: huffed
Following righting reflex: tail shake
- 37 Slight brown nasal staining; slight brown staining head;
slight lack of grooming back; slightly emaciated
- 38 Slight brown staining head; slight lack of grooming back;
slightly emaciated
In the arena: limited walking
Touch response: appears to be retching
During manipulations: slightly hunched (noted at end of
observations); vocalising moderately and slightly awkward
to handle
- 39 Slight lack of grooming back
- 40 Slight lack of grooming back

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 4 males, 5000 ppm				
Animal no	96	97	98	99	100
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	Y
degree					2
IN THE ARENA					
Tremors	B1	B1	B1		
Grooming	N	N	N	N	N
Activity count	1	1	3	4	1
Arousal	2	2	3	4	2
Rearing count	0	1	4	4	1
Bolus count	3	0	0	0	0
Urine present	N	N	S	N	N
Gait	U	U	U		U
MANIPULATIONS					
Approach	5	3	3	5	1
Touch	4	5	3	4	1
vocalises					
degree					
Startle	3	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	2	2	6
turns		1	1	2	
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.7	37.9	37.1	37.3	37.2
Bodyweight (g)	460	410	461	430	412
GRIP STRENGTH (kg) #					
Forelimb	1.68	1.79	1.53	1.73	1.84
Hindlimb	1.26	1.43	1.51	1.23	1.64
FOOTSPRAY (cm) #					
	10.0	14.5	8.1	10.4	8.4

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 96 Slight brown nasal staining
In the arena: sitting facing into corner
During temperature: slight body tremors
- 97 Slight lack of grooming back; red staining around right eye;
slightly emaciated
In the arena: sitting in corner
- 98 Slight lack of grooming back; slight piloerection
In the arena: eyes partially to half-closed occasionally;
limited walking
During temperature: slight body tremors
- 99 Marked hairloss forepaws; slight brown nasal staining;
slight lack of grooming back
- 100 Slight hairloss left forelimb; slight lack of grooming
back; top left tooth broken
In the arena: sat facing into corner
During manipulations: vocalising moderately

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 1 females, Control				
Animal no	46	47	48	49	50
OBSERVATIONS					
IN THE CAGE					
Posture	R	dead	S	S	S
IN THE HAND					
Removing	2		2	2	2
Handling	2		2	2	2
Salivation	N		N	N	N
degree					
Vocalising	N		N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	Y		Y	N	N
Activity count	19		15	4	18
Arousal	4		4	4	4
Rearing count	17		17	1	16
Bolus count	0		0	0	0
Urine present	N		S	N	N
Gait	T1				T1
MANIPULATIONS					
Approach	5		3	5	3
Touch	5		1	3	2
vocalises					
degree					
Startle	3		3	3	2
Righting reflex	1		1	1	1
Tail pinch	3		3	3	3
turns					
vocalises	Y		Y	Y	Y
degree	1		2	2	1
Pupil reflex	B		B	B	B
Temperature (°C)	39.2		38.9	37.2	38.6
Bodyweight (g)	266		240	236	215
GRIP STRENGTH (kg) #					
Forelimb	1.02		1.37	0.83	0.90
Hindlimb	1.09		1.41	0.58	0.55
FOOTSPLAY (cm) #	6.3		10.4	4.7	7.2

Values represent the mean of two trials

Additional comments

Animal no.

- 46 Slight lack of grooming back; slight brown staining head
- 48 Slight brown staining ears
- 49 Right lower tooth pale
- 50 Slight lack of grooming back; slight brown staining head, neck, ears

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 1 females, Control					
	Animal no	101	102	103	104	105
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	R	S	R
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	Y	N	N
degree				2		
IN THE ARENA						
Tremors						
Grooming		Y	Y	Y	N	Y
Activity count		17	8	11	7	29
Arousal		4	4	4	4	4
Rearing count		27	9	13	8	13
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait		T2				T2
MANIPULATIONS						
Approach		3	3	3	3	3
Touch		3	2	3	3	3
vocalises						
degree						
Startle		3	3	3	2	3
Righting reflex		1	2	1	1	1
Tail pinch		2	3	3	3	3
turns		2		1	1	
vocalises		Y	Y	Y	Y	
degree		1	2	2	2	
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.8	38.5	38.3	38.8	38.4
Bodyweight (g)		276	286	311	258	275
GRIP STRENGTH (kg) #						
Forelimb		0.99	1.33	1.41	0.99	1.17
Hindlimb		0.76	1.24	1.50	1.06	1.38
FOOTSPLAY (cm) #						
		5.7	11.4	12.2	8.0	9.2

Values represent the mean of two trials

Additional comments

Animal no.

- 101 Slight brown nasal staining
Grip strength: hindlimb trial 1 not gripping bar
- 102 Slight brown staining head
- 103 Slight brown nasal staining; slight brown staining head
In the arena: whole body groom
- 104 Slight brown nasal staining; slight lack of grooming back
- 105 Moderate hairloss forelimbs

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 2 females, 200 ppm				
Animal no	56	57	58	59	60
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	N	Y	N
Activity count	5	5	8	18	20
Arousal	4	4	4	4	4
Rearing count	3	4	9	16	20
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait				T1	
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	5	3	3	5
vocalises					
degree					
Startle	3	2	3	2	2
Righting reflex	1	1	1	1	1
Tail pinch	2	3	3	3	2
turns	2	1	1		1
vocalises	Y	Y	Y	Y	Y
degree	2	1	1	1	2
Pupil reflex	B	B	B	B	R
Temperature (°C)	38.3	37.3	39.2	38.9	38.8
Bodyweight (g)	307	254	274	247	256
GRIP STRENGTH (kg) #					
Forelimb	1.43	0.98	0.99	0.64	1.17
Hindlimb	1.10	0.81	0.84	1.04	1.32
FOOTSPLAY (cm) #	6.2	6.7	7.9	6.6	8.7

Values represent the mean of two trials

Additional comments

Animal no.

- 57 Slightly emaciated; slight brown nasal staining
During manipulations: feet cold
- 58 Slight brown staining head; slight kink near end of tail
- 59 Slight lack of grooming back; slight brown staining head
- 60 Slight brown staining head, ears; moderate hairloss
forelimbs; slight lack of grooming rump
Pupil reflex: left pupil dilated

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 2 females, 200 ppm				
Animal no	106	107	108	109	110
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	N
degree		2	1		
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	N
Activity count	4	17	22	12	25
Arousal	4	4	4	4	4
Rearing count	10	13	20	15	9
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait		T1	T1		
MANIPULATIONS					
Approach	5	3	3	3	3
Touch	4	1	3	3	4
vocalises					
degree					
Startle	2	2	3	3	2
Righting reflex	1	1	1	1	1
Tail pinch	2	2	2	6	2
turns	1	2	2		2
vocalises	Y	Y	Y	Y	Y
degree	2	1	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.0	38.1	38.2	38.3	38.5
Bodyweight (g)	221	275	306	288	275
GRIP STRENGTH (kg) #					
Forelimb	0.61	1.33	1.24	1.12	1.03
Hindlimb	0.79	1.49	1.42	1.25	0.92
FOOTSPLAY (cm) #	7.0	7.6	10.6	8.4	9.6

Values represent the mean of two trials

Additional comments

Animal no.

- 107 Slight brown staining head, ears
- 108 Slight brown nasal staining
- In the arena: tail curling occasionally
- 109 Slight lack of grooming back
- 110 Slight brown nasal staining

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 3 females, 1000 ppm				
Animal no	66	67	68	69	70
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	Y	Y	N
degree			1	1	
IN THE ARENA					
Tremors					
Grooming	N	Y	N	Y	Y
Activity count	4	18	13	5	18
Arousal	4	4	4	4	4
Rearing count	5	15	17	2	9
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait		T1			T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	5	3	3	1
vocalises					
degree					
Startle	4	4	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns			1		
vocalises	Y	Y	Y	Y	Y
degree	1	2	2	2	2
Pupil reflex	B	B	B	N	B
Temperature (°C)	37.9	38.7	38.7	38.7	38.1
Bodyweight (g)	232	283	203	225	261
GRIP STRENGTH (kg) #					
Forelimb	1.30	1.21	1.00	0.94	1.20
Hindlimb	0.58	1.40	0.93	1.45	1.14
FOOTSPLAY (cm) #	5.4	11.2	6.1	11.8	10.6

Values represent the mean of two trials

Additional comments

Animal no.

- 66 Slight brown staining head
 68 During grip strength: slightly awkward to handle and vocalising moderately
 69 Slightly emaciated
 In the arena: whole body grooming
 Prior to tail pinch: head shake
 Pupil reflex: both pupils constricted
 During manipulations: slightly awkward to handle
 70 Slight brown staining head, neck; slight lack of grooming rump

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 3 females, 1000 ppm					
	Animal no	111	112	113	114	115
OBSERVATIONS						
IN THE CAGE						
Posture		S	R	R	S	R
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	Y	Y	N
degree				1	1	
IN THE ARENA						
Tremors		B1				
Grooming		N	N	N	N	N
Activity count		1	9	15	29	1
Arousal		3	4	4	4	2
Rearing count		4	11	15	19	1
Bolus count		0	0	0	0	0
Urine present		S	N	N	N	N
Gait		U			T2	
MANIPULATIONS						
Approach		3	6	3	3	6
Touch		3	3	5	3	3
vocalises				Y		
degree				1		
Startle		3	3	3	2	3
Righting reflex		1	1	1	1	1
Tail pinch		3	3	6	3	2
turns			1			3
vocalises		Y	Y	Y	Y	Y
degree		2	2	3	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.9	37.5	38.8	39.5	38.8
Bodyweight (g)		303	246	307	218	243
GRIP STRENGTH (kg) #						
Forelimb		1.23	1.55	1.47	1.12	0.97
Hindlimb		1.40	0.97	1.33	1.20	0.97
FOOTSPRAY (cm) #						
		10.7	9.6	9.0	6.7	6.6

Values represent the mean of two trials

Additional comments

Animal no.

- 111 Slight brown staining head; slight lack of grooming back
In the arena: sitting/rearing in corner
- 112 Slight brown nasal staining; slight brown staining head;
slight lack of grooming back
- 113 Slight brown staining head, ears; slight brown nasal
staining
During manipulations: slightly awkward to handle and
vocalising moderately
- 114 Slightly emaciated
- 115 Slight brown nasal staining
In the arena: sitting in corner

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 4 females, 5000 ppm				
Animal no	76	77	78	79	80
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors				B1	
Grooming	Y	Y	N	N	Y
Activity count	4	10	9	4	18
Arousal	4	4	4	3	4
Rearing count	5	9	4	2	15
Bolus count	0	0	0	0	0
Urine present	N	N	N	S	N
Gait	HU1A1	T2			T2
MANIPULATIONS					
Approach	3	3	2	3	3
Touch	2	3	3	4	3
vocalises					
degree					
Startle	3	2	2	3	2
Righting reflex	2	1	1	1	1
Tail pinch	3	3	6	6	3
turns		1		1	
vocalises		Y	Y	Y	Y
degree		2	3	2	2
Pupil reflex	B	B	B	R	B
Temperature (°C)	38.7	38.9	38.4	37.9	38.8
Bodyweight (g)	234	259	250	243	220
GRIP STRENGTH (kg) #					
Forelimb	0.24	1.22	1.37	1.14	1.30
Hindlimb	0.49	0.93	0.36	1.04	1.16
FOOTSPLAY (cm) #	5.6	5.9	6.4	6.1	6.7

Values represent the mean of two trials

Additional comments

Animal no.

- 76 Slight lack of grooming back; slightly emaciated
During righting reflex: abdomen appears slightly swollen
- 77 Lower teeth white in colour
- 78 Slight brown staining head, neck; moderate brown staining
ears; slight lack of grooming back; top left tooth broken
Grip strength: not gripping bar with hindfeet
During grip strength and foot splay: slightly awkward to
handle
- 79 Pupil reflex: left pupil dilated to the light

APPENDIX 4

(Functional observational battery - continued)

Week 8	Group 4 females, 5000 ppm				
Animal no	116	117	118	119	120
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	N	N	Y	N
degree	1			2	
IN THE ARENA					
Tremors					
Grooming	N	Y	Y	Y	Y
Activity count	8	22	9	18	17
Arousal	4	4	4	4	4
Rearing count	10	23	11	18	15
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T1			
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	5	2	5	3	3
vocalises					
degree					
Startle	3	2	2	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	6	3	3	2
turns	1				2
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.6	38.6	38.5	38.8	38.4
Bodyweight (g)	204	238	279	252	261
GRIP STRENGTH (kg) #					
Forelimb	1.05	1.02	1.26	1.07	0.93
Hindlimb	0.73	1.35	1.12	1.22	1.17
FOOTSPRAY (cm) #	5.6	10.9	11.5	7.1	9.3

Values represent the mean of two trials

Additional comments

Animal no.

- 116 Slight brown staining head; slightly emaciated
- 117 Moderate hairloss forelimbs; slight brown staining head
During grip strength and foot splay: moderately awkward to handle and vocalising moderately
- 119 Slight lack of grooming back; slightly emaciated
In the arena: tail curling occasionally
- 120 In the arena: tail curling occasionally; scratching
Following tail pinch: moderately awkward to handle and vocalising moderately

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 1 males, Control					
	Animal no	6	7	8	9	10
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	S	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	N	N	Y
degree						1
IN THE ARENA						
Tremors		B1	B1	B1		
Grooming		N	N	N	N	N
Activity count		4	1	5	10	5
Arousal		3	2	4	4	4
Rearing count		4	0	3	11	3
Bolus count		0	0	0	0	0
Urine present		N	S	N	S	N
Gait			U			
MANIPULATIONS						
Approach		3	2	3	3	5
Touch		4	1	3	1	1
vocalises						
degree						
Startle		3	3	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		3	3	3	2	3
turns					3	1
vocalises		Y	Y	Y	Y	Y
degree		2	2	1	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.6	37.6	36.5	37.1	38.4
Bodyweight (g)		574	549	600	583	562
GRIP STRENGTH (kg) #						
Forelimb		1.90	1.02	1.73	1.57	1.13
Hindlimb		1.27	1.67	1.66	1.07	1.18
FOOTSPRAY (cm) #						
		10.1	8.2	9.8	7.05	10.5

Values represent the mean of two trials

Additional comments

Animal no.

- 6 Moderate lack of grooming back; slight hairloss left side of thorax
In the arena: head shake
During manipulations: head shake
During temperature: slight body tremors, head shake
- 7 Moderate lack of grooming back
In the arena: sitting in corner
- 8 Slight lack of grooming back; moderate hairloss left forepaw; slight brown nasal staining
In the arena: eyes partially to half-closed occasionally:
- 9 Slight brown nasal staining; slight lack of grooming back
- 10 Slight brown nasal staining; slight lack of grooming back
During temperature: slight body tremors

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 1 males, Control				
Animal no	81	82	83	84	85
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	R	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	Y
degree					1
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	Y
Activity count	7	4	8	11	5
Arousal	4	4	4	4	4
Rearing count	5	7	2	10	4
Bolus count	0	0	0	0	0
Urine present	S	N	N	S	S
Gait			O	Alt1	
MANIPULATIONS					
Approach	3	2	2	3	5
Touch	1	4	1	3	1
vocalises					
degree					
Startle	3	3	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	6	2	2	6
turns			3	2	
vocalises	Y	Y	Y	Y	Y
degree	2	2	3	2	2
Pupil reflex	B	L	B	B	B
Temperature (°C)	37.2	37.9	37.6	37.8	37.0
Bodyweight (g)	566	505	533	508	516
GRIP STRENGTH (kg) #					
Forelimb	1.49	1.50	0.71	0.78	1.70
Hindlimb	1.17	1.73	0.9	1.29	1.52
FOOTSPRAY (cm) #					
	6.45	13.1	11.4	7.3	11.6

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 81 Moderate lack of grooming back
- 82 Slight brown nasal staining; slight lack of grooming dorsum
Pupil reflex: right pupil constricted
During manipulations: toenail damaged on left hind foot
- 83 Slight lack of grooming back, rump; slight brown staining
neck, head; top teeth slightly crooked
During manipulations: toenail damaged left forepaw; markedly
awkward to handle and attempting to bite handler
throughout
- 84 Moderate lack of grooming dorsum; slight brown nasal
staining; slight brown staining head
In the arena: tail elevated occasionally
- 85 Slight brown nasal staining; moderate lack of grooming back
In the arena: tail curling occasionally

APPENDIX 4

(Functional observational battery - continued)

Week 13		Group 2 males, 200 ppm				
	Animal no	16	17	18	19	20
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	S	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	5	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	Y	Y	Y	N
degree			3	1	2	
IN THE ARENA						
Tremors		B1				
Grooming		N	N	Y	N	N
Activity count		4	8	11	5	15
Arousal		3	4	4	4	4
Rearing count		1	1	11	2	10
Bolus count		0	0	0	0	0
Urine present		N	N	S	S	N
Gait		A1				
MANIPULATIONS						
Approach		2	2	3	3	3
Touch		4	4	3	1	1
vocalises			Y			
degree			2			
Startle		3	3	3	3	3
Righting reflex		2	1	1	1	1
Tail pinch		2	6	6	5	3
turns		2				
vocalises		Y	Y	Y	Y	Y
degree		3	3	2	3	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.7	39.1	38.1	37.2	37.2
Bodyweight (g)		552	628	458	523	588
GRIP STRENGTH (kg) #						
Forelimb		1.16	1.99	1.58	1.40	1.57
Hindlimb		1.73	1.56	1.51	1.14	1.46
FOOTSPRAY (cm) #		8.8	12.8	7.1	7.9	10.9

Values represent the mean of two trials

Additional comments

Animal no.

- 16 Moderate lack of grooming dorsum
Righting reflex: slow to complete
Tail pinch: position stationary
- 17 Marked lack of grooming dorsum
During manipulations: vocalising moderately
- 18 Slight lack of grooming back
- 19 Slight hairloss and scabs right forelimb; slight lack of grooming back
- 20 Slight lack of grooming back
During manipulations: faeces soft

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 2 males, 200 ppm				
Animal no	86	87	88	89	90
OBSERVATIONS					
IN THE CAGE					
Posture	S	R	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	N	Y	Y	Y
degree	2		1	2	2
IN THE ARENA					
Tremors			B1		B1
Grooming	N	Y	Y	N	N
Activity count	7	7	2	1	4
Arousal	4	4	4	2	4
Rearing count	6	8	2	0	3
Bolus count	0	0	0	0	0
Urine present	S	N	N	S	N
Gait			U	U	U
MANIPULATIONS					
Approach	5	5	3	1	3
Touch	4	1	1	0	1
vocalises					
degree					
Startle	4	3	3	2	3
Righting reflex	1	1	2	1	1
Tail pinch	6	2	3	3	3
turns		2			
vocalises	Y	Y	Y	Y	Y
degree	3	1	1	1	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.0	37.5	37.1	38.0	37.9
Bodyweight (g)	445	591	544	580	568
GRIP STRENGTH (kg) #					
Forelimb	1.18	1.39	1.65	1.49	1.50
Hindlimb	1.47	1.12	1.41	1.25	0.91
FOOTSPLAY (cm) #	8.4	8.8	10.5	6.0	10.4

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 86 Top teeth crooked; top left tooth broken; top right tooth white; slight brown nasal staining; slightly emaciated; slight lack of grooming back
Touch response: "huffs"
- 87 Slight lack of grooming dorsum
Righting reflex: slow to complete
- 88 Slight lack of grooming back
In the arena: eyes partially to half-closed occasionally; sitting in corner
- 89 Moderate lack of grooming dorsum; slight brown nasal staining
In the arena: sitting in corner
Touch response: "huffs"
- 90 Moderate brown nasal staining; slight brown staining head, neck; slight lack of grooming dorsum
In the arena: tail curling occasionally; limited walking
Righting reflex: slow to complete

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 3 males, 1000 ppm					
	Animal no	26	27	28	29	30
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	R	R
IN THE HAND						
Removing		2	2	2	2	3
Handling		2	3	2	2	2
Salivation		N	N	N	N	Y
degree						1
Vocalising		N	Y	N	Y	Y
degree			2		2	1
IN THE ARENA						
Tremors		B1		B1		
Grooming		Y	N	N	N	N
Activity count		4	7	7	8	8
Arousal		3	4	4	4	3
Rearing count		3	6	5	8	6
Bolus count		0	0	0	0	0
Urine present		S	N	N	N	N
Gait						Alt1
MANIPULATIONS						
Approach		5	3	2	3	1
Touch		1	4	1	1	4
vocalises			Y			Y
degree			2			2
Startle		2	4	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		5	2	6	3	5
turns			3			
vocalises		Y	Y	Y		Y
degree		2	2	2		2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.7	38.5	37.8	37.3	38.1
Bodyweight (g)		527	580	539	502	527
GRIP STRENGTH (kg) #						
Forelimb		1.29	1.72	1.68	1.75	1.40
Hindlimb		1.11	1.62	1.3	1.39	1.39
FOOTSPLAY (cm) #						
		5.7	14.2	12.9	9.0	7.0

Values represent the mean of two trials

Additional comments

Animal no.

- 26 Moderate lack of grooming dorsum; slight hairloss right forelimb
- 27 Moderate lack of grooming back; slight brown nasal staining; tip of tail kinked
- Touch response: spontaneous vocalisation
- 28 Slight lack of grooming dorsum
- In the arena: movement of hindlimbs appeared slow and jerky occasionally
- 29 Moderate lack of grooming dorsum
- 30 Moderate lack of grooming dorsum
- Startle response: vocalising moderately
- During manipulations: vocalising loudly

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 3 males, 1000 ppm				
Animal no	91	92	93	94	95
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	R	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	5	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	Y	N	Y
degree		3	2		2
IN THE ARENA					
Tremors		B1			B1
Grooming	N	N	N	N	N
Activity count	3	4	9	10	1
Arousal	3	3	4	4	2
Rearing count	5	1	3	2	2
Bolus count	0	0	0	0	0
Urine present	S	S	N	N	N
Gait	U				U
MANIPULATIONS					
Approach	5	2	2	2	2
Touch	1	4	1	1	3
vocalises					
degree					
Startle	3	4	3	3	2
Righting reflex	1	1	1	1	1
Tail pinch	2	6	3	3	5
turns	2				
vocalises	Y	Y	Y		Y
degree	2	2	1		2
Pupil reflex	B	B	B	B	B
Temperature (°C)	36.8	38.7	38.2	36.8	36.4
Bodyweight (g)	503	579	562	556	546
GRIP STRENGTH (kg) #					
Forelimb	1.50	0.97	1.86	1.06	1.26
Hindlimb	1.49	0.88	1.73	1.56	1.19
FOOTSPLAY (cm) #					
	9.0	11.4	11.6	9.75	8.2

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 91 Slight brown nasal staining; slight brown staining head;
moderate lack of grooming back
In the arena: limited walking
- 92 Moderate lack of grooming back, rump
Touch response: "huffs"
During manipulations: faeces soft
- 93 Slight brown nasal staining; slight lack of grooming back
- 94 Slight lack of grooming back, rump; moderate brown nasal
staining
During temperature: head shake
- 95 Slight lack of grooming back
In the arena: sitting in corner
Righting reflex: slow to complete

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 4 males, 5000 ppm				
Animal no	36	37	38	39	40
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	5	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	Y	N	Y	Y	Y
degree	2		2	2	2
IN THE ARENA					
Tremors					B1
Grooming	N	N	N	N	N
Activity count	1	11	3	2	2
Arousal	3	4	3	3	3
Rearing count	3	6	3	0	0
Bolus count	1	0	0	0	0
Urine present	N	N	N	N	S
Gait	U		U	U	U
MANIPULATIONS					
Approach	5	3	0	5	1
Touch	1	4	4	3	0
vocalises					Y
degree					3
Startle	3	2	3	3	4
Righting reflex	1	1	1	1	1
Tail pinch	3	5	3	2	3
turns				2	
vocalises	Y	Y	Y	Y	Y
degree	2	1	3	3	3
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.5	37.8	38.2	37.5	37.6
Bodyweight (g)	514	451	457	480	549
GRIP STRENGTH (kg) #					
Forelimb	1.96	1.34	1.2	0.98	1.74
Hindlimb	1.31	1.59	1.19	1.51	1.23
FOOTSPRAY (cm) #					
	11.5	7.8	11.8	6.9	14.1

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 36 Slight lack of grooming back
In the arena: sitting in corner
- 37 Moderate lack of grooming dorsum; slight brown staining
head; slight brown nasal staining
- 38 Moderate lack of grooming back; slight brown staining ears
In the arena: limited walking
Approach response: "huffs"
During manipulations: vocalising loudly
- 39 Slight lack of grooming back
In the arena: eyes partially closed occasionally; sitting
in corner
- 40 Slight brown nasal staining; slight lack of grooming dorsum
In the arena: sitting in corner
Touch response: huffing and then froze
During manipulations: head shake

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 4 males, 5000 ppm				
Animal no	96	97	98	99	100
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	Y	N	N	N
degree		2			
IN THE ARENA					
Tremors	B1				
Grooming	N	N	Y	Y	N
Activity count	1	8	6	6	2
Arousal	2	4	4	4	3
Rearing count	1	11	5	5	5
Bolus count	5	0	0	0	0
Urine present	S	N	S	N	S
Gait	U		Alt1		U
MANIPULATIONS					
Approach	5	2	3	5	2
Touch	1	3	3	4	1
vocalises					
degree					
Startle	2	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	5	3
turns				1	
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.5	37.6	37.4	37.4	37.1
Bodyweight (g)	476	452	533	491	400
GRIP STRENGTH (kg) #					
Forelimb	1.88	1.76	1.61	1.39	1.56
Hindlimb	1.51	1.55	1.45	1.26	1.73
FOOTSPRAY (cm) #					
	10.4	14.7	8.8	10.4	8.7

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 96 Red staining around right eye; moderate lack of grooming
ventrum; slight brown nasal staining; right top tooth
missing
In the arena: eyes partially closed occasionally; sat facing
edge of arena
During temperature: slight body tremors
- 97 Slight brown nasal staining; top teeth crooked; red
staining right eye; slight lack of grooming back
In the arena: tail curling occasionally
- 98 Slight lack of grooming dorsum
In the arena: head shake; eyes slightly to partially closed
occasionally
- 99 Moderate lack of grooming back, rump; moderate brown nasal
staining; slight brown staining head; marked hairloss
forepaws
- 100 Moderate lack of grooming back; top teeth crooked; red
staining around right eye; slightly emaciated
In the arena: initial slipping of hindlimbs; eyes partially
to half-closed occasionally; sitting in corner

APPENDIX 4

(Functional observational battery - continued)

Week 13		Group 1 females, Control				
	Animal no	46	47	48	49	50
OBSERVATIONS						
IN THE CAGE						
Posture		S	DEAD	S	S	S
IN THE HAND						
Removing		2		2	2	2
Handling		2		2	2	2
Salivation		N		N	N	N
degree						
Vocalising		N		N	N	N
degree						
IN THE ARENA						
Tremors						
Grooming		Y		N	N	N
Activity count		7		10	16	16
Arousal		4		4	4	4
Rearing count		2		17	9	15
Bolus count		0		0	0	0
Urine present		N		N	N	N
Gait					T1	
MANIPULATIONS						
Approach		5		3	3	2
Touch		3		1	3	3
vocalises						
degree						
Startle		4		2	3	3
Righting reflex		1		1	1	1
Tail pinch		3		3	3	3
turns						
vocalises		Y		Y		Y
degree		2		2		2
Pupil reflex		B		B	B	B
Temperature (°C)		38.4		38.7	37.9	38.4
Bodyweight (g)		282		260	238	238
GRIP STRENGTH (kg) #						
Forelimb		1.16		1.48	0.78	1.13
Hindlimb		1.28		1.41	0.29	0.77
FOOTSPRAY (cm) #		6.6		9.6	5.9	7.8

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 46 Slight lack of grooming back
In the arena: walking backwards
- 48 Top teeth crooked; top right tooth broken; slight lack of grooming back; slight brown nasal staining
In the arena: head shake
- 49 Slight lack of grooming back
In the arena: scratching
During grip strength: not gripping with hindlimbs
During temperature: vocalising softly
- 50 Slight brown staining head, neck; moderate brown staining ears; slight lack of grooming back
In the arena: gait slightly swaying occasionally

APPENDIX 4

(Functional observational battery - continued)

Week 13		Group 1 females, Control				
	Animal no	101	102	103	104	105
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	R	S	R
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	3	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		Y	Y	Y	N	N
degree		1	2	1		
IN THE ARENA						
Tremors			B1	B1		
Grooming		N	Y	Y	N	Y
Activity count		13	2	4	2	15
Arousal		4	3	3	3	4
Rearing count		10	4	5	7	8
Bolus count		0	0	0	0	0
Urine present		N	N	S	N	N
Gait		T2	U		U	
MANIPULATIONS						
Approach		3	5	3	3	3
Touch		4	1	3	2	3
vocalises						
degree						
Startle		3	2	3	3	3
Righting reflex		2	1	1	1	1
Tail pinch		3	3	3	3	3
turns						
vocalises		Y	Y	Y	Y	Y
degree		2	2	2	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.6	37.3	37.9	38.1	38.3
Bodyweight (g)		295	319	348	268	318
GRIP STRENGTH (kg) #						
Forelimb		1.28	1.48	1.39	1.09	1.09
Hindlimb		1.40	1.41	1.44	1.31	1.30
FOOTSPRAY (cm) #		7.6	11.7	12.9	10.2	9.5

Values represent the mean of two trials

Additional comments

Animal no.

- 101 Slight lack of grooming back; slight brown nasal staining
- 102 Slight brown nasal staining; moderate brown staining ears
In the arena: sitting in corner
- 103 Slight lack of grooming back; slight brown staining head
In the arena: whole body grooming
- 104 Slight brown nasal staining, slight brown staining head,
neck; slight lack of grooming dorsum
In the arena: sitting in corner
- 105 Slight lack of grooming dorsum; moderate hairloss forelimbs

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 2 females, 200 ppm				
Animal no	56	57	58	59	60
OBSERVATIONS					
IN THE CAGE					
Posture	R	S	S	S	S
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	N	N	N	N	Y
Activity count	4	8	9	20	16
Arousal	3	4	4	4	4
Rearing count	4	5	4	9	13
Bolus count	0	0	0	0	0
Urine present	N	N	N	S	N
Gait		T1			
MANIPULATIONS					
Approach	3	3	3	5	3
Touch	3	3	3	3	5
vocalises					
degree					
Startle	3	2	2	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	2
turns			1		2
vocalises	Y	Y	Y		Y
degree	2	1	1		2
Pupil reflex	B	B	B	B	R
Temperature (°C)	38.1	37.5	38.5	38.9	38.5
Bodyweight (g)	336	275	303	263	280
GRIP STRENGTH (kg) #					
Forelimb	1.61	0.98	1.03	0.56	1.30
Hindlimb	1.05	1.12	0.99	1.08	1.40
FOOTSPLAY (cm) #	9.5	8.1	8.3	7.8	8.9

Values represent the mean of two trials

Additional comments

Animal no.

- 56 Slight brown staining head; neck; slight lack of grooming back
- 57 Slight lack of grooming dorsum
- 58 Slight lack of grooming dorsum; slight brown staining head
- 59 Slight brown staining head, ears; slight lack of grooming back
- 60 Slight brown staining head, neck; marked hairloss and slight brown staining forelimbs; slight brown staining ears
Pupil reflex: left pupil dilated to light

APPENDIX 4

(Functional observational battery - continued)

Week 13		Group 2 females, 200 ppm				
	Animal no	106	107	108	109	110
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	S	R
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	N	N	N
degree						
IN THE ARENA						
Tremors		N	N	Y	Y	Y
Grooming		N	N	Y	Y	Y
Activity count		5	16	6	13	15
Arousal		4	4	4	4	4
Rearing count		6	12	5	13	8
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait			T2			
MANIPULATIONS						
Approach		3	3	3	3	3
Touch		1	1	3	3	3
vocalises						
degree						
Startle		2	3	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		2	2	3	6	3
turns		2	2	1		
vocalises		Y	Y	Y	Y	Y
degree		2	2	2	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		38.3	38.3	38.3	39	38.1
Bodyweight (g)		239	296	348	314	300
GRIP STRENGTH (kg) #						
Forelimb		0.66	0.92	1.62	1.30	1.24
Hindlimb		0.69	1.27	1.24	1.17	1.05
FOOTSPLAY (cm) #		7.6	7.2	10.3	7.0	10.3

Values represent the mean of two trials

Additional comments

Animal no.

- 106 Moderate lack of grooming dorsum
In the arena: walking backwards
- 107 Slight brown staining head, neck, ears, and shoulders;
slight brown nasal staining; slightly emaciated
- 108 Slight lack of grooming back
In the arena: tail curling occasionally; whole body grooming
- 109 Moderate lack of grooming dorsum; slight brown staining ears
In the arena: limp in left leg noted occasionally
- 110 Slight lack of grooming back; slight brown nasal staining

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 3 females, 1000 ppm				
Animal no	66	67	68	69	70
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	3	2
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors					
Grooming	Y	Y	Y	Y	Y
Activity count	7	19	18	11	14
Arousal	4	4	4	4	4
Rearing count	6	13	15	8	12
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait	T1				T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	5	3	3	3
vocalises					
degree					
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	2	3	6	3
turns		2			1
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	38.0	38.6	38.4	38.6
Bodyweight (g)	257	316	314	259	270
GRIP STRENGTH (kg) #					
Forelimb	1.33	1.30	0.87	0.69	1.12
Hindlimb	1.26	1.33	0.91	1.73	0.85
FOOTSPLAY (cm) #	7.7	11.3	6.1	11.7	9.8

Values represent the mean of two trials

Additional comments

Animal no.

- 66 Slight brown staining head, neck; slight hairloss forelimbs
- 67 Slight lack of grooming dorsum
- 68 Slight lack of grooming dorsum
During grip strength: slightly awkward to handle
During temperature: vocalising moderately
- 69 Slight lack of grooming dorsum
During grip strength: not gripping with left forelimb
vocalising moderately; slightly awkward to handle
During manipulations: toenail on left hindlimb damaged
- 70 Slight lack of grooming dorsum; slight brown staining head, neck

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 3 females, 1000 ppm					
	Animal no	111	112	113	114	115
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	R	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	3	3	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		Y	Y	Y	N	N
degree		1	2	2		
IN THE ARENA						
Tremors						B1-2
Grooming		N	N	N	N	N
Activity count		1	13	6	23	1
Arousal		2	4	4	4	2
Rearing count		1	12	6	13	0
Bolus count		0	0	0	0	0
Urine present		N	M	N	N	N
Gait		U			T1	U
MANIPULATIONS						
Approach		3	6	3	3	5
Touch		3	4	3	1	3
vocalises						
degree						
Startle		3	2	3	3	3
Righting reflex		1	1	1	1	1
Tail pinch		3	6	3	3	3
turns			1			1
vocalises		Y	Y	Y	Y	Y
degree		2	2	1	2	2
Pupil reflex		B	B	B	B	B
Temperature (°C)		37.6	37.8	38.8	39.3	37.2
Bodyweight (g)		318	256	337	234	266
GRIP STRENGTH (kg) #						
Forelimb		1.35	1.62	1.54	1.34	1.02
Hindlimb		1.46	1.47	0.96	1.31	0.79
FOOTSPRAY (cm) #						
		8.7	9.6	9.8	7.3	7.1

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 111 Top teeth slightly crooked; slight lack of grooming dorsum
In the arena: eyes slightly closed occasionally; sat
in the middle of square in the arena
- 112 Moderate lack of grooming dorsum; slightly patchy hairloss
rump; slight brown nasal staining
During manipulations: developed a wet urogenital region
During grip strength and footsplay: slightly awkward to
handle and vocalising moderately
- 113 Slight lack of grooming back; slight brown staining head,
neck; slight brown nasal staining; moderate brown staining
ears
During pupil reflex and grip strength: moderately awkward
to handle and vocalising moderately
- 114 Slight lack of grooming back; slight brown staining head,
neck
- 115 Slight lack of grooming dorsum
In the arena: eyes partially to fully closed occasionally;
sitting in corner; tremors progressed from slight to
moderate

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 4 females, 5000 ppm					
	Animal no	76	77	78	79	80
OBSERVATIONS						
IN THE CAGE						
Posture		S	S	S	S	S
IN THE HAND						
Removing		2	2	2	2	2
Handling		2	2	2	2	2
Salivation		N	N	N	N	N
degree						
Vocalising		N	N	Y	N	Y
degree				1		2
IN THE ARENA						
Tremors						
Grooming		N	Y	N	N	Y
Activity count		1	6	12	1	16
Arousal		2	4	4	2	4
Rearing count		2	7	8	0	11
Bolus count		0	0	0	0	0
Urine present		N	N	N	N	N
Gait		U			U	
MANIPULATIONS						
Approach		3	3	5	3	3
Touch		2	3	3	4	5
vocalises						
degree						
Startle		3	2	2	3	2
Righting reflex		1	1	1	1	1
Tail pinch		3	3	2	2	3
turns		1		2	2	
vocalises		Y	Y	Y	Y	Y
degree		2	2	2	2	2
Pupil reflex		B	B	B	R	B
Temperature (°C)		39.0	38.1	37.9	37.0	38.7
Bodyweight (g)		279	285	262	267	240
GRIP STRENGTH (kg)#						
Forelimb		0.89	1.24	0.80	1.44	1.18
Hindlimb		0.84	0.97	0.71	1.34	1.15
FOOTSPRAY (cm) #						
		5.1	9.2	7.5	6.3	7.6

Values represent the mean of two trials

Additional comments

Animal no.

- 76 Slight lack of grooming back
In the arena: sitting in corner
- 77 Slight lack of grooming dorsum; bottom teeth white
- 78 Slight lack of grooming dorsum; moderate brown staining ears; slight brown staining head, neck; slight hairloss hindlimbs
- During grip strength: moderately awkward to handle
- 79 Slight lack of grooming dorsum; slight brown staining head
In the arena: sitting in corner
Pupil reflex: left pupil dilated
- 80 Slight lack of grooming dorsum; slight brown staining head
During grip strength: vocalising moderately

APPENDIX 4

(Functional observational battery - continued)

Week 13	Group 4 females, 5000 ppm				
Animal no	116	117	118	119	120
OBSERVATIONS					
IN THE CAGE					
Posture	S	S	S	S	R
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	N	N	N
degree					
Vocalising	N	N	N	N	N
degree					
IN THE ARENA					
Tremors		B1			
Grooming	N	Y	N	Y	N
Activity count	8	11	6	14	15
Arousal	4	4	4	4	4
Rearing count	5	7	8	14	11
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait		T2		T1	
MANIPULATIONS					
Approach	3	3	5	3	3
Touch	3	1	2	3	3
vocalises					
degree					
Startle	3	2	2	3	3
Righting reflex	1	1	1	1	1
Tail pinch	6	3	3	3	6
turns					
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.9	38.1	38.1	38.5	38.3
Bodyweight (g)	213	257	312	268	287
GRIP STRENGTH (kg) #					
Forelimb	1.20	0.84	1.48	1.16	1.21
Hindlimb	0.77	1.46	1.39	1.34	1.24
FOOTSPLAY (cm) #	4.8	8.6	13.0	6.2	10.4

Values represent the mean of two trials

APPENDIX 4

(Functional observational battery - continued)

Additional comments

Animal no.

- 116 Slightly emaciated; slight lack of grooming back; slight brown staining ears, head, neck
- 117 Slightly emaciated; moderate brown staining ears; slight brown staining head, neck; marked hairloss forelimbs
During grip strength, foot splay and temperature: slightly awkward to handle and vocalising moderately
- 118 Slight lack of grooming back; slight brown staining head, neck; slight brown nasal staining; moderate brown staining ears
- 119 Slight lack of grooming back; slight brown staining head, neck; slight brown nasal staining
In the arena: tail curling occasionally
- 120 Slight brown nasal staining; slightly emaciated
In the arena: tail curling occasionally

APPENDIX 5

Locomotor activity

Animal no.	Total time spent in locomotor activity (secs) when tested during:			
	Pre-dose	Week 4	Week 8	Week 13
Group 1♂ Control				
6	300	306	436	298
7	411	407	485	414
8	529	748	1127	886
9	359	627	706	480
10	480	606	993	920
81	403	783	898	543
82	463	1168	1098	965
83	304	473	458	361
84	348	983	1185	571
85	415	1090	1038	937
Group 2♂ 200 ppm				
16	507	536	834	771
17	353	360	444	352
18	468	886	841	833
19	375	526	914	970
20	298	744	830	606
86	166	866	823	681
87	556	742	568	416
88	195	670	1243	457
89	145	401	658	462
90	474	1268	1163	728
Group 3♂ 1000 ppm				
26	253	468	768	322
27	429	622	744	818
28	351	610	941	572
29	419	576	609	486
30	370	498	739	438
91	208	464	902	553
92	348	985	965	924
93	587	1259	1094	512
94	211	848	829	643
95	481	649	518	311
Group 4♂ 5000 ppm				
36	534	833	509	414
37	314	830	418	252
38	610	1237	1118	851
39	197	553	741	477
40	461	676	1078	830
96	158	437	502	329
97	138	824	918	741
98	586	1009	1421	1121
99	175	445	585	417
100	210	646	522	650

APPENDIX 5

(Locomotor activity - continued)

Animal no.	Total time spent in locomotor activity (secs) when tested during:			
	Pre-dose	Week 4	Week 8	Week 13
Group 1♀ Control				
46	469	845	941	746
47	329	933	dead	
48	496	828	919	448
49	390	1068	766	631
50	293	685	719	445
101	499	1049	1151	777
102	475	1199	732	679
103	562	776	1290	618
104	260	849	763	547
105	380	1735	1819	1447
Group 2♀ 200 ppm				
56	563	848	671	359
57	266	917	648	364
58	387	1039	847	390
59	508	950	1392	985
60	651	1398	1502	1163
106	365	536	857	435
107	483	1051	1457	1013
108	648	1187	1110	1046
109	436	904	979	1004
110	348	809	750	617
Group 3♀ 1000 ppm				
66	917	1667	1368	1001
67	418	1078	694	654
68	1203	1501	1904	1255
69	235	808	379	408
70	543	1440	1114	768
111	205	513	468	251
112	291	1273	2061	1266
113	344	848	920	576
114	373	1201	1021	871
115	330	696	618	327
Group 4♀ 5000 ppm				
76	137	333	267	420
77	641	1381	1203	1109
78	532	1277	1053	1138
79	535	665	916	488
80	410	743	564	442
116	444	657	684	265
117	523	1164	1251	842
118	315	557	674	407
119	642	778	1435	878
120	423	1354	1265	619

N-(n-butyl) thiophosphoric triamide (NBPT)
TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS
INCORPORATING A NEUROTOXICITY SCREEN

Volume 2

Sponsor

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APPENDIX 6

Ophthalmoscopy - individual observations

Pre-dose (3 June 1996)

Group	No. of rats examined	Rat no.	Observation
1♂	15		No abnormalities detected
2♂	15		No abnormalities detected
3♂	15		No abnormalities detected
4♂	15		No abnormalities detected
1♀	15	77	No abnormalities detected
2♀	15		No abnormalities detected
3♀	15		No abnormalities detected
4♀	15		Bilateral persistent pupillary membranes

x

APPENDIX 6

(Ophthalmoscopy - continued)

Week 13 (3, 4 September 1996)

Group	No. of rats examined	Rat no.	Observation
1♂	15	9	Right eye, intravitreal haemorrhage
4♂	15	32	Right eye, chromodacryorrhoea (slight)
		36	Right eye, hyper-reflectivity (retinal)
		96	Right eye, chromodacryorrhoea
1♀	14		No abnormalities detected
4♀	15		No abnormalities detected

APPENDIX 7

Haematology - individual values

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	PCV Hb		RBC	MCHC	MCV	MCH	WBC	N	L	E	B	M
		%	g/dl	10 ¹² /l	g/dl	fl	pg	Total 10 ⁹ /l					
1♂ Control	1	48.1	16.0	8.52	33.3	56.4	18.8	17.54	1.62	15.25	0.13	0.07	0.24
	2	47.8	15.7	7.74	32.9	61.8	20.4	11.33	1.23	9.41	0.14	0.06	0.25
	3	47.2	15.8	8.47	33.4	55.7	18.6	13.73	1.66	11.44	0.12	0.05	0.21
	4	47.2	16.4	8.00	34.7	59.0	20.5	18.09	2.51	14.86	0.10	0.09	0.29
	5	47.0	15.8	8.01	33.5	58.7	19.7	20.37	2.81	17.02	0.21	0.08	0.13
	6	45.2	15.1	8.20	33.4	55.1	18.4	12.69	1.15	11.26	0.09	0.03	0.12
	7	48.3	16.3	8.68	33.8	55.7	18.8	10.31	1.26	8.54	0.10	0.03	0.22
	8	43.4	14.7	8.47	34.0	51.2	17.4	15.99	1.98	13.65	0.07	0.05	0.14
	9	48.9	16.4	8.66	33.6	56.5	19.0	15.88	1.40	14.19	0.07	0.05	0.11
	10	44.5	15.3	7.84	34.4	56.8	19.6	17.90	2.17	14.90	0.20	0.07	0.21
	Mean sd	46.8 1.80	15.8 0.57	8.26 0.345	33.7 0.54	56.7 2.79	19.1 0.95	15.38 3.265	1.78 0.572	13.05 2.756	0.12 0.049	0.06 0.020	0.19 0.063
2♂ 200	11	46.5	16.2	8.18	34.9	56.8	19.8	11.24	1.96	8.89	0.06	0.02	0.19
	12	46.9	15.8	8.25	33.8	56.8	19.2	19.22	2.78	15.88	0.12	0.06	0.25
	13	48.1	16.4	8.34	34.1	57.7	19.7	14.65	1.46	12.61	0.14	0.06	0.22
	14	47.1	15.9	8.46	33.8	55.7	18.8	12.58	1.63	10.21	0.23	0.04	0.27
	15	44.7	15.2	7.91	34.0	56.4	19.2	13.33	1.85	10.77	0.13	0.04	0.29
	16	47.6	16.6	8.46	34.9	56.3	19.7	11.62	1.69	9.55	0.08	0.03	0.17
	17	45.4	15.8	7.99	34.8	56.9	19.8	10.81	1.48	8.44	0.08	0.03	0.21
	18	46.4	16.0	8.28	34.4	56.0	19.3	12.43	1.97	10.02	0.09	0.04	0.21
	19	47.9	16.4	8.79	34.3	54.4	18.7	17.05	1.33	15.02	0.10	0.06	0.22
	20	47.3	16.2	8.56	34.2	55.2	18.9	12.50	1.09	10.85	0.10	0.03	0.24
	Mean sd	46.8 1.08	16.1 0.40	8.32 0.263	34.3 0.42	56.2 0.94	19.3 0.42	13.54 2.698	1.72 0.465	11.22 2.513	0.11 0.048	0.04 0.014	0.23 0.036

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
1♂ Control	1	0.23	-	-	-	-	-	-	-	-	-	968	23
	2	0.23	-	-	-	-	-	-	-	-	-	737	21
	3	0.25	-	-	-	-	-	-	-	-	-	988	25
	4	0.24	-	-	-	-	-	-	-	-	-	1093	25
	5	0.13	-	-	-	+	-	-	-	-	-	963	24
	6	0.03	-	-	-	+	-	-	-	-	-	977	22
	7	0.14	-	-	-	+	-	-	-	-	-	1070	22
	8	0.09	-	+	-	+	-	-	-	-	-	1229	25
	9	0.05	-	-	-	-	-	-	-	-	-	1275	24
	10	0.36	-	-	-	+	-	-	-	-	-	1094	22
	Mean sd	0.18 0.104										1039 151.7	23 1.5
2♂ 200	11	0.12	-	-	-	-	-	-	-	-	-	993	25
	12	0.14	-	-	-	+	-	-	-	-	-	1260	21
	13	0.15	-	-	-	-	-	-	-	-	-	1036	23
	14	0.21	-	-	-	-	-	-	-	-	-	1209	21
	15	0.25	-	-	-	-	-	-	-	-	-	1324	23
	16	0.10	-	-	-	+	-	-	-	-	-	1021	25
	17	0.16	-	-	-	-	-	-	-	-	-	933	21
	18	0.11	-	-	-	+	-	-	-	-	-	950	22
	19	0.32	-	-	-	-	-	-	-	-	-	985	ctd
	20	0.19	-	-	-	-	-	-	-	-	-	1412	23
	Mean sd	0.18 0.069										1112 172.8	23 1.5

sd Standard deviation

ctd Clotted sample

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	PCV Hb		RBC	MCHC	MCV	MCH	WBC Total	N	L	E	B	M
		%	g/dl	$10^{12}/l$	g/dl	fl	pg	$10^9/l$	$10^9/l$	$10^9/l$	$10^9/l$	$10^9/l$	$10^9/l$
3 δ 1000	21	46.9	16.4	8.28	34.9	56.6	19.8	18.36	1.54	16.28	0.07	0.07	0.17
	22	44.7	15.4	7.77	34.4	57.6	19.8	16.06	2.05	13.20	0.26	0.04	0.23
	23	46.7	16.3	8.31	34.9	56.2	19.6	11.87	1.02	10.30	0.06	0.03	0.25
	24	48.0	16.5	8.44	34.4	56.9	19.6	12.95	2.63	9.65	0.11	0.04	0.30
	25	45.2	15.7	8.63	34.7	52.4	18.1	15.88	1.74	13.70	0.07	0.04	0.13
	26	47.6	16.1	8.32	33.9	57.1	19.4	14.33	1.28	12.47	0.15	0.03	0.17
	27	44.5	15.4	7.53	34.6	59.1	20.4	15.29	1.37	13.30	0.14	0.05	0.19
	28	47.7	16.2	8.47	33.9	56.3	19.1	11.64	1.20	9.90	0.14	0.02	0.19
	29	44.0	15.7	7.88	35.5	55.9	19.9	16.46	2.44	13.48	0.11	0.04	0.18
	30	47.6	16.5	8.55	34.8	55.6	19.3	9.89	1.05	8.40	0.03	0.02	0.17
	Mean	46.3	16.0	8.22	34.6	56.4	19.5	14.27	1.63	12.07	0.11	0.04	0.20
	sd	1.53	0.43	0.366	0.48	1.71	0.61	2.624	0.571	2.415	0.065	0.015	0.049
4 δ 5000	31	47.6	16.5	9.03	34.6	52.7	18.2	15.68	2.35	12.80	0.11	0.06	0.17
	32	45.5	15.8	8.36	34.6	54.4	18.9	13.14	1.56	11.16	0.09	0.04	0.17
	33	46.4	16.6	8.77	35.8	52.9	18.9	13.50	1.39	11.61	0.10	0.04	0.21
	34	49.2	17.0	9.08	34.5	54.2	18.7	12.15	0.90	10.90	0.07	0.03	0.14
	35	44.9	15.6	8.43	34.8	53.3	18.6	10.49	1.88	8.16	0.16	0.04	0.15
	36	47.5	16.2	8.46	34.2	56.2	19.2	11.12	1.00	9.67	0.07	0.02	0.20
	37	49.3	16.7	9.41	33.9	52.4	17.8	10.08	0.94	8.78	0.09	0.04	0.13
	38	45.4	16.0	8.57	35.2	52.9	18.6	14.80	1.58	12.81	0.10	0.03	0.14
	39	45.0	15.6	8.12	34.6	55.5	19.2	11.12	1.71	9.13	0.07	0.03	0.09
	40	45.0	15.6	8.36	34.7	53.8	18.7	12.50	1.45	10.51	0.07	0.03	0.17
	Mean	46.6	16.2	8.66	34.7	53.8	18.7	12.46	1.48	10.55	0.09	0.04	0.16
	sd	1.72	0.52	0.403	0.52	1.26	0.43	1.845	0.454	1.613	0.028	0.011	0.035

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
3♂ 1000	21	0.23	-	-	-	+	-	-	-	-	-	1104	23
	22	0.29	-	-	-	+	-	-	-	-	-	1148	22
	23	0.21	-	-	-	-	-	-	-	-	-	987	22
	24	0.22	-	-	-	-	-	-	-	-	-	950	22
	25	0.19	-	-	-	+	-	-	-	-	-	1081	25
	26	0.22	-	-	-	+	-	-	-	-	-	887	23
	27	0.25	-	-	-	-	-	-	-	-	-	1337	23
	28	0.19	-	-	-	-	-	-	-	-	-	984	20
	29	0.21	-	-	-	+	-	-	-	-	-	1145	23
	30	0.21	-	-	-	-	-	-	-	-	-	1149	21
	Mean	0.22										1077	22
	sd	0.030										130.0	1.5
4♂ 5000	31	0.19	-	-	-	+	-	-	-	-	-	1112	23
	32	0.12	-	-	-	+	-	-	-	-	-	1208	21
	33	0.16	-	-	-	+	-	-	-	-	-	916	23
	34	0.11	-	-	-	+	-	-	-	-	-	955	21
	35	0.09	-	-	-	-	-	-	-	-	-	1130	25
	36	0.15	-	-	-	-	-	-	-	-	-	938	24
	37	0.11	-	-	-	-	-	-	-	-	-	1252	23
	38	0.15	-	-	-	-	-	-	-	-	-	1314	25
	39	0.08	-	-	-	+	-	-	-	-	-	1048	24
	40	0.26	-	-	-	+	-	-	-	-	-	918	22
	Mean	0.14										1079	23
	sd	0.053										146.9	1.5

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg	WBC Total $10^9/l$	N $10^9/l$	L $10^9/l$	E $10^9/l$	B $10^9/l$	M $10^9/l$
1♀ Control	41	47.0	16.5	8.38	35.1	56.1	19.7	12.48	1.98	10.03	0.07	0.04	0.23
	42	45.9	15.9	8.20	34.6	55.9	19.3	10.29	2.10	7.62	0.15	0.03	0.22
	43	45.1	15.9	7.95	35.1	56.8	19.9	9.27	1.23	7.61	0.08	0.01	0.14
	44	46.0	16.1	8.25	35.0	55.7	19.5	8.46	1.03	7.03	0.09	0.02	0.19
	45	45.1	15.7	8.14	34.8	55.4	19.3	6.51	1.08	5.14	0.05	0.01	0.12
	46	47.0	16.5	8.58	35.1	54.7	19.2	9.47	1.35	7.69	0.08	0.02	0.18
	47	47.9	15.9	8.16	33.1	58.7	19.5	2.93	0.35	2.48	0.04	0.00	0.02
	48	44.4	15.8	8.26	35.5	53.7	19.1	8.88	0.52	8.11	0.08	0.02	0.10
	49	42.8	15.5	8.03	36.1	53.4	19.3	9.94	1.24	8.33	0.09	0.02	0.16
	50	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	Mean	45.7	16.0	8.22	34.9	55.6	19.4	8.69	1.21	7.12	0.08	0.02	0.15
	sd	1.55	0.34	0.186	0.81	1.61	0.25	2.680	0.578	2.156	0.031	0.012	0.065
2♀ 200	51	44.9	15.8	8.21	35.1	54.8	19.2	13.07	1.78	10.56	0.11	0.04	0.31
	52	46.2	16.3	8.47	35.4	54.6	19.3	11.20	1.94	8.88	0.09	0.03	0.16
	53	44.4	15.6	8.07	35.1	55.1	19.3	9.28	0.83	8.13	0.08	0.02	0.12
	54	42.9	15.5	7.91	36.1	54.3	19.6	11.86	1.45	10.05	0.13	0.02	0.11
	55	44.0	15.4	8.18	35.0	53.8	18.8	7.74	0.65	6.92	0.03	0.01	0.06
	56	46.6	15.9	8.19	34.1	57.0	19.4	8.61	1.54	6.68	0.12	0.01	0.17
	57	46.5	16.5	8.18	35.4	56.9	20.1	10.11	0.74	9.05	0.07	0.02	0.14
	58	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	59	44.7	15.3	8.26	34.2	54.1	18.5	8.59	1.38	6.92	0.12	0.02	0.07
	60	43.8	15.7	7.94	35.9	55.1	19.8	8.64	0.53	7.77	0.11	0.02	0.13
	Mean	44.9	15.8	8.16	35.1	55.1	19.3	9.90	1.20	8.33	0.10	0.02	0.14
	sd	1.30	0.40	0.169	0.67	1.15	0.48	1.790	0.524	1.406	0.032	0.009	0.073

sd Standard deviation

ctd Clotted sample

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
1♀ Control	41	0.13	-	-	-	-	-	-	-	-	-	903	22
	42	0.17	-	-	-	-	-	-	-	-	-	963	20
	43	0.20	-	-	-	-	-	-	-	-	-	1029	20
	44	0.10	-	-	-	-	-	-	-	-	-	873	ctd
	45	0.11	-	-	-	-	-	-	-	-	-	604	20
	46	0.14	-	-	-	-	-	-	-	-	-	1124	ctd
	47	0.02	-	-	-	-	-	-	-	-	-	804	ctd
	48	0.06	-	-	-	-	-	-	-	-	-	1044	ctd
	49	0.09	-	-	-	-	-	-	-	-	-	1035	18
	50	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	21
	Mean sd	0.11 0.055										931 157.6	20 1.3
2♀ 200	51	0.27	-	-	-	-	-	-	-	-	-	914	21
	52	0.09	-	-	-	-	-	-	-	-	-	1042	19
	53	0.10	-	-	-	-	-	-	-	-	-	981	19
	54	0.10	-	-	-	-	-	-	-	-	-	1101	19
	55	0.06	-	-	-	-	-	-	-	-	-	1161	19
	56	0.09	-	-	-	-	-	-	-	-	-	1002	18
	57	0.09	-	-	-	-	-	-	-	-	-	1213	20
	58	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	59	0.09	-	-	-	-	-	-	-	-	-	1057	19
	60	0.09	-	-	-	-	-	-	-	-	-	896	18
	Mean sd	0.11 0.062										1041 106.1	19 0.9

sd Standard deviation

ctd Clotted sample

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg	WBC Total $10^9/l$	N $10^9/l$	L $10^9/l$	E $10^9/l$	B $10^9/l$	M $10^9/l$
3♀ 1000	61	45.1	15.9	7.81	35.2	57.8	20.3	6.59	0.46	5.90	0.04	0.01	0.13
	62	44.8	15.6	8.05	34.9	55.6	19.4	7.06	0.42	6.44	0.04	0.01	0.09
	63	48.7	16.8	8.78	34.5	55.5	19.1	10.89	0.69	9.89	0.07	0.02	0.12
	64	46.5	15.8	8.18	33.9	56.9	19.3	10.22	1.27	8.55	0.07	0.03	0.21
	65	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	66	46.7	16.2	8.61	34.7	54.3	18.8	4.76	0.54	4.12	0.04	0.01	0.03
	67	43.3	15.0	7.59	34.7	57.0	19.8	8.23	0.69	7.27	0.13	0.03	0.05
	68	45.3	15.8	8.24	34.9	54.9	19.2	9.74	1.13	8.33	0.06	0.01	0.13
	69	45.8	16.2	8.59	35.3	53.3	18.8	8.94	0.99	7.66	0.09	0.02	0.08
	70	42.1	15.1	7.38	36.0	57.0	20.5	10.89	1.76	8.85	0.04	0.03	0.10
	Mean	45.4	15.8	8.14	34.9	55.8	19.5	8.59	0.88	7.45	0.06	0.02	0.10
	sd	1.93	0.56	0.480	0.58	1.48	0.61	2.116	0.444	1.749	0.030	0.009	0.052
4♀ 5000	71	45.7	16.2	8.61	35.4	53.1	18.8	8.89	1.09	7.50	0.09	0.02	0.11
	72	45.4	16.1	8.39	35.5	54.1	19.2	7.60	0.85	6.47	0.09	0.02	0.10
	73	44.5	15.7	7.96	35.4	55.9	19.8	6.95	1.70	5.02	0.10	0.01	0.08
	74	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	75	42.9	14.7	7.63	34.3	56.2	19.2	8.74	0.29	8.18	0.08	0.03	0.10
	76	44.1	15.3	7.94	34.6	55.5	19.2	8.84	0.64	7.91	0.15	0.01	0.07
	77	44.3	15.6	7.79	35.1	56.8	20.0	8.38	1.07	7.01	0.04	0.02	0.15
	78	44.3	15.1	7.48	34.0	59.2	20.2	7.79	1.63	5.97	0.07	0.02	0.06
	79	42.1	15.3	7.43	36.2	56.7	20.5	6.33	0.95	5.12	0.05	0.02	0.08
	80	43.7	15.6	8.37	35.7	52.2	18.7	7.47	0.92	6.25	0.11	0.01	0.13
	Mean	44.1	15.5	7.96	35.1	55.5	19.5	7.89	1.02	6.60	0.09	0.02	0.10
	sd	1.12	0.47	0.422	0.71	2.12	0.64	0.898	0.442	1.141	0.033	0.007	0.029

sd Standard deviation

ctd Clotted sample

APPENDIX 7

(Haematology - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
3♀ 1000	61	0.06	-	-	-	-	-	-	-	-	-	1052	20
	62	0.06	-	-	-	-	-	-	-	-	-	1259	18
	63	0.09	-	-	-	-	-	-	-	-	-	1121	21
	64	0.09	-	-	-	-	-	-	-	-	-	1111	21
	65	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	23
	66	0.03	-	-	-	-	-	-	-	-	-	922	21
	67	0.06	-	-	-	-	-	-	-	-	-	1252	ctd
	68	0.09	-	-	-	-	-	-	-	-	-	949	19
	69	0.10	-	-	-	-	-	-	-	-	-	954	20
	70	0.11	-	-	-	-	-	-	-	-	-	944	ctd
	Mean	0.08										1063	20
	sd	0.025										131.7	1.5
4♀ 5000	71	0.08	-	-	-	-	-	-	-	-	-	1133	20
	72	0.07	-	-	-	-	-	-	-	-	-	1042	21
	73	0.04	-	-	-	-	-	-	-	-	-	1206	ctd
	74	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
	75	0.07	-	-	-	-	-	-	-	-	-	1148	18
	76	0.05	-	-	-	-	-	-	-	-	-	1243	20
	77	0.09	-	-	-	-	-	-	-	-	-	883	21
	78	0.05	-	-	-	-	-	-	-	-	-	1346	19
	79	0.09	-	-	-	-	-	-	-	-	-	909	ctd
	80	0.05	-	-	-	-	-	-	-	-	-	1229	18
	Mean	0.07										1127	19
	sd	0.019										155.3	1.3

sd Standard deviation

ctd Clotted sample

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg	WBC Total $10^9/l$	N $10^9/l$	L $10^9/l$	E $10^9/l$	B $10^9/l$	M $10^9/l$
1♂ Control	1	43.9	15.9	8.17	36.3	53.8	19.5	13.18	1.67	10.83	0.20	0.03	0.25
	2	50.4	16.0	8.96	31.7	56.3	17.8	10.57	1.24	8.80	0.06	0.04	0.25
	3	51.2	16.9	9.81	33.0	52.2	17.2	9.24	1.29	7.58	0.09	0.01	0.14
	4	48.4	16.3	8.73	33.6	55.5	18.6	13.55	1.47	11.54	0.15	0.04	0.23
	5	47.4	15.2	8.73	32.1	54.3	17.4	16.61	1.98	13.99	0.29	0.05	0.16
	6	48.6	16.0	9.42	33.0	51.6	17.0	13.67	1.27	11.87	0.20	0.04	0.20
	7	49.7	16.3	9.58	32.9	51.9	17.1	12.47	1.47	10.18	0.21	0.03	0.37
	8	50.2	16.6	10.24	33.1	49.1	16.3	13.41	2.47	10.44	0.15	0.05	0.20
	9	51.5	17.0	9.80	32.9	52.6	17.3	16.05	1.26	14.16	0.16	0.06	0.23
	10	47.0	15.6	9.04	33.1	51.9	17.2	15.37	1.69	13.15	0.12	0.04	0.22
	Mean sd	48.8 2.30	16.2 0.56	9.25 0.630	33.2 1.22	52.9 2.10	17.5 0.90	13.41 2.298	1.58 0.394	11.25 2.147	0.16 0.066	0.04 0.014	0.23 0.062
2♂ 200	11	49.5	16.5	9.13	33.3	54.2	18.1	12.07	1.40	10.43	0.08	0.02	0.09
	12	49.7	16.6	9.64	33.4	51.6	17.2	19.26	2.99	15.53	0.23	0.08	0.26
	13	51.3	16.9	9.43	32.9	54.4	17.9	13.37	1.36	11.57	0.18	0.04	0.17
	14	49.0	16.2	9.68	33.2	50.6	16.8	12.12	2.04	9.18	0.32	0.03	0.31
	15	46.7	15.8	9.25	33.8	50.5	17.1	10.51	1.51	8.43	0.18	0.02	0.24
	16	50.8	16.9	9.49	33.3	53.5	17.8	12.60	1.71	10.29	0.21	0.02	0.21
	17	50.0	16.5	9.57	33.0	52.2	17.2	11.71	1.42	9.78	0.14	0.03	0.19
	18	48.9	16.1	9.00	33.0	54.4	17.9	11.89	1.25	10.14	0.12	0.04	0.20
	19	51.0	16.7	9.65	32.8	52.9	17.4	14.54	2.22	11.87	0.08	0.06	0.16
	20	52.5	17.3	10.08	32.9	52.1	17.1	9.38	0.97	8.05	0.11	0.03	0.15
	Mean sd	49.9 1.60	16.6 0.44	9.49 0.311	33.2 0.30	52.6 1.48	17.5 0.44	12.75 2.688	1.69 0.587	10.53 2.135	0.17 0.075	0.04 0.019	0.20 0.062

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
1♂ Control	1	0.20	-	-	-	+++	-	-	-	-	-	876	22
	2	0.18	+	-	-	+++	-	-	-	-	-	1043	23
	3	0.12	-	-	-	+	-	-	-	-	-	1009	26
	4	0.13	-	-	-	+	-	-	-	-	-	1138	23
	5	0.13	-	-	-	+++	-	-	-	-	nr	1044	24
	6	0.11	-	+	-	+++	-	-	-	-	-	987	23
	7	0.21	-	-	-	+++	-	-	-	-	-	1113	22
	8	0.11	++	++	-	+	-	-	-	-	-	1277	24
	9	0.18	-	-	-	+	-	-	-	-	-	1210	24
	10	0.16	-	-	-	+++	-	-	-	-	-	1119	21
	Mean sd	0.15 0.038										1082 115.3	23 1.4
2♂ 200	11	0.05	-	-	-	-	-	-	-	-	-	935	25
	12	0.17	+	+	-	+++	-	+	-	-	-	1195	24
	13	0.07	-	-	-	+	-	-	-	-	-	944	24
	14	0.24	-	+	-	+	-	-	-	-	-	1210	22
	15	0.13	-	-	-	+++	-	-	-	-	-	1265	23
	16	0.15	-	-	-	+	-	-	-	-	-	933	26
	17	0.14	-	-	-	+++	-	-	-	-	-	1122	22
	18	0.15	-	-	-	+++	-	-	+	-	-	1208	24
	19	0.15	-	-	-	+++	-	-	-	-	-	1087	24
	20	0.07	-	-	-	+	-	-	-	-	-	1376	27
	Mean sd	0.13 0.056										1128 152.3	24 1.5

sd Standard deviation

nr Not recorded

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	PCV Hb		RBC 10 ¹² /l	MCHC g/dl	MCV fl	MCH pg	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l
		%	g/dl										
3♂ 1000	21	46.9	15.4	8.86	32.8	52.9	17.4	14.08	1.41	12.35	0.08	0.06	0.09
	22	45.8	15.1	8.68	33.0	52.8	17.4	17.03	2.60	13.46	0.40	0.06	0.26
	23	48.3	16.2	9.38	33.5	51.5	17.2	10.19	1.57	8.15	0.12	0.02	0.21
	24	49.3	16.7	9.28	33.8	53.1	18.0	13.44	1.72	11.13	0.17	0.04	0.23
	25	48.5	15.7	9.65	32.4	50.3	16.3	15.08	1.98	12.65	0.18	0.04	0.12
	26	50.8	16.7	9.33	32.8	54.5	17.9	14.74	1.52	12.62	0.17	0.05	0.21
	27	45.6	15.6	8.11	34.2	56.2	19.2	16.02	2.11	13.39	0.16	0.04	0.18
	28	50.4	16.9	9.91	33.5	50.9	17.1	9.66	1.25	8.05	0.13	0.02	0.15
	29	46.0	15.7	8.73	34.2	52.6	18.0	16.63	1.52	14.65	0.14	0.06	0.14
	30	50.9	17.4	9.66	34.2	52.7	18.0	9.34	1.05	7.90	0.11	0.02	0.14
	Mean	48.3	16.1	9.16	33.4	52.8	17.7	13.62	1.67	11.44	0.17	0.04	0.17
	sd	2.09	0.75	0.553	0.66	1.70	0.77	2.903	0.452	2.513	0.088	0.017	0.054
4♂ 5000	31	48.8	16.1	9.70	33.0	50.3	16.6	17.19	1.50	15.22	0.13	0.06	0.14
	32	48.6	16.2	9.03	33.4	53.9	18.0	13.84	4.52	8.92	0.07	0.03	0.16
	33	47.7	16.1	9.33	33.7	51.2	17.2	15.30	1.33	13.58	0.18	0.05	0.09
	34	51.1	17.6	10.00	34.4	51.1	17.6	13.87	2.13	11.25	0.15	0.04	0.16
	35	47.7	15.8	9.26	33.2	51.5	17.1	9.77	1.18	8.07	0.19	0.02	0.19
	36	48.4	15.9	9.11	32.9	53.1	17.5	11.53	1.40	9.64	0.12	0.03	0.19
	37	51.0	17.2	10.48	33.8	48.7	16.4	12.52	1.32	10.79	0.14	0.04	0.14
	38	46.2	15.3	9.13	33.1	50.6	16.7	14.85	1.57	12.81	0.14	0.06	0.14
	39	47.8	16.2	8.98	33.9	53.2	18.0	10.61	1.02	9.29	0.10	0.03	0.08
	40	48.7	16.2	9.81	33.2	49.7	16.5	12.31	1.18	10.75	0.09	0.03	0.12
	Mean	48.6	16.3	9.48	33.5	51.3	17.2	13.18	1.72	11.03	0.13	0.04	0.14
	sd	1.50	0.67	0.496	0.48	1.65	0.60	2.271	1.031	2.251	0.038	0.014	0.037

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
3♂ 1000	21	0.08	-	-	-	+	-	-	-	-	-	1130	24
	22	0.25	-	+	-	+++	-	-	-	-	-	1104	23
	23	0.12	-	-	-	+	-	-	-	-	-	1180	25
	24	0.15	-	-	-	+++	-	-	-	-	-	1070	24
	25	0.11	+	+	-	+	-	-	-	-	-	1122	24
	26	0.17	-	-	-	+	-	-	-	-	-	954	24
	27	0.14	-	-	-	+++	-	-	-	-	-	1330	23
	28	0.06	-	+	-	+	-	-	-	-	-	1102	23
	29	0.14	-	-	-	+++	-	-	-	-	-	1239	23
	30	0.11	-	-	-	+	-	-	-	-	-	1246	23
	Mean	0.13										1148	24
	sd	0.053										105.8	0.8
4♂ 5000	31	0.14	-	+	-	+	-	-	-	-	-	1112	23
	32	0.13	-	-	-	+	-	-	-	-	-	1094	22
	33	0.07	-	-	-	+	-	-	-	-	-	990	23
	34	0.13	+	+	-	+++	-	-	-	-	-	995	23
	35	0.11	-	-	-	-	-	-	-	-	-	1230	22
	36	0.14	-	-	-	+	-	-	-	-	-	937	23
	37	0.09	-	+	-	+	-	-	-	-	-	1256	25
	38	0.13	-	-	-	+	-	-	-	-	-	1358	24
	39	0.09	-	-	-	+	-	-	-	-	-	1013	24
	40	0.14	-	+	-	+++	-	-	-	-	-	1138	23
	Mean	0.12										1112	23
	sd	0.025										135.5	0.9

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	PCV Hb		RBC 10 ¹² /l	MCHC g/dl	MCV fl	MCH pg	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l
		%	g/dl										
1♀ Control	41	48.4	16.6	8.83	34.4	54.8	18.8	8.10	0.70	7.18	0.07	0.02	0.09
	42	50.1	16.4	8.96	32.7	56.0	18.3	6.26	0.60	5.38	0.12	0.02	0.07
	43	47.4	16.0	8.53	33.7	55.6	18.7	5.94	0.75	4.87	0.12	0.00	0.13
	44	47.6	16.0	8.71	33.7	54.7	18.4	8.52	0.84	7.34	0.10	0.01	0.14
	45	46.4	15.4	8.54	33.3	54.3	18.1	5.96	0.58	5.05	0.10	0.01	0.13
	46	49.6	16.7	9.15	33.6	54.2	18.2	6.27	0.71	5.38	0.04	0.01	0.09
	48	45.1	15.1	8.31	33.5	54.3	18.2	8.71	0.66	7.64	0.17	0.02	0.14
	49	46.1	15.2	8.47	33.1	54.4	18.0	7.30	0.56	6.50	0.09	0.01	0.10
	50	48.5	16.4	8.96	33.8	54.1	18.3	8.78	1.46	6.94	0.15	0.02	0.15
	Mean	47.7	16.0	8.72	33.5	54.7	18.3	7.32	0.76	6.25	0.11	0.01	0.12
	sd	1.64	0.61	0.276	0.48	0.66	0.26	1.229	0.276	1.083	0.039	0.007	0.028
2♀ 200	51	47.9	16.2	8.61	33.9	55.6	18.8	8.96	1.07	7.52	0.09	0.01	0.18
	52	48.0	16.3	8.97	34.0	53.5	18.2	8.11	0.47	7.41	0.08	0.02	0.09
	53	46.6	15.4	8.56	33.1	54.4	18.0	8.52	0.82	7.31	0.14	0.02	0.16
	54	45.3	15.4	8.29	34.0	54.6	18.6	10.51	0.81	9.39	0.10	0.02	0.11
	55	45.3	14.5	8.34	32.0	54.3	17.3	5.55	0.51	4.88	0.06	0.00	0.06
	56	48.5	16.0	8.85	32.9	54.8	18.0	8.95	1.09	7.31	0.22	0.02	0.21
	57	48.8	16.3	8.61	33.3	56.7	18.9	11.47	0.93	10.16	0.10	0.03	0.16
	58	48.3	15.7	8.23	32.6	58.7	19.1	6.25	0.73	5.29	0.05	0.01	0.12
	59	46.1	15.3	8.55	33.1	53.9	17.9	6.52	0.87	5.45	0.07	0.01	0.08
	60	44.0	14.6	8.05	33.3	54.7	18.2	7.19	0.93	6.08	0.08	0.01	0.07
	Mean	46.9	15.6	8.51	33.2	55.1	18.3	8.20	0.82	7.08	0.10	0.02	0.12
	sd	1.65	0.66	0.283	0.64	1.54	0.55	1.884	0.208	1.730	0.049	0.008	0.051

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
1 ♀ Control	41	0.04	-	-	-	-	-	-	-	-	-	1028	23
	42	0.07	-	-	-	-	-	-	-	-	-	1005	20
	43	0.07	-	-	-	-	-	-	-	-	-	1057	22
	44	0.08	-	-	-	-	-	-	-	-	-	836	20
	45	0.08	-	-	-	-	-	-	-	-	-	728	20
	46	0.05	-	-	-	-	-	-	-	-	-	1173	19
	48	0.08	-	-	-	-	-	-	-	-	-	1126	21
	49	0.03	-	-	-	-	-	-	-	-	-	992	21
	50	0.06	-	-	-	-	-	-	-	-	-	988	20
	Mean	0.06										993	21
	sd	0.019										137.1	1.2
2 ♀ 200	51	0.08	-	-	-	-	-	-	-	-	-	971	21
	52	0.04	-	-	-	-	-	-	-	-	-	1125	21
	53	0.09	-	-	-	-	-	-	-	-	-	990	20
	54	0.07	-	-	-	-	-	-	-	-	-	1079	20
	55	0.03	-	-	-	-	-	-	-	-	-	1122	19
	56	0.10	-	-	-	-	-	-	-	-	-	1009	20
	57	0.08	-	-	-	-	-	-	-	-	-	1195	19
	58	0.06	-	-	-	-	-	-	-	-	-	920	19
	59	0.05	-	-	-	-	-	-	-	-	-	999	20
	60	0.04	-	-	-	-	-	-	-	-	-	1040	20
	Mean	0.06										1045	20
	sd	0.024										84.0	0.7

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	PCV Hb		RBC	MCHC	MCV	MCH	WBC	N	L	E	B	M
		%	g/dl	10 ¹² /l	g/dl	fl	pg	Total 10 ⁹ /l					
3♀ 1000	61	47.8	16.0	8.65	33.6	55.2	18.5	5.94	0.64	5.11	0.05	0.00	0.08
	62	45.3	15.1	8.14	33.3	55.7	18.6	7.04	0.67	6.20	0.04	0.02	0.08
	63	50.3	17.2	9.24	34.2	54.4	18.6	10.08	0.65	9.22	0.06	0.02	0.09
	64	47.2	15.9	8.54	33.7	55.2	18.6	8.23	1.04	6.90	0.12	0.02	0.10
	65	47.5	16.0	8.93	33.8	53.2	17.9	9.74	1.73	7.52	0.14	0.02	0.22
	66	46.9	15.8	8.90	33.8	52.7	17.8	7.42	0.84	6.30	0.10	0.01	0.09
	67	46.6	15.2	8.30	32.7	56.2	18.4	6.65	0.70	5.76	0.08	0.02	0.05
	68	47.5	15.8	8.71	33.2	54.5	18.1	8.00	0.79	6.88	0.11	0.02	0.13
	69	48.8	16.5	9.26	33.8	52.8	17.8	9.30	1.28	7.73	0.09	0.02	0.10
	70	46.9	15.9	8.25	33.9	56.8	19.3	8.57	0.51	7.84	0.07	0.02	0.10
	Mean sd	47.5 1.34	15.9 0.60	8.69 0.395	33.6 0.43	54.7 1.42	18.4 0.47	8.10 1.360	0.89 0.371	6.95 1.187	0.09 0.032	0.02 0.007	0.10 0.046
	4♀ 5000	71	45.9	15.6	8.52	34.0	53.9	18.3	7.80	0.92	6.53	0.20	0.01
72		46.9	15.8	8.76	33.7	53.6	18.0	7.57	1.27	6.09	0.09	0.01	0.07
73		46.4	16.0	8.54	34.5	54.4	18.7	5.91	0.76	4.95	0.07	0.01	0.08
74		44.3	14.9	8.15	33.7	54.3	18.3	6.86	0.90	5.67	0.11	0.02	0.11
75		42.0	14.1	7.79	33.5	54.0	18.1	7.27	0.47	6.48	0.11	0.01	0.12
76		45.1	15.4	8.38	34.2	53.9	18.4	7.43	0.66	6.42	0.13	0.02	0.14
77		49.1	16.4	8.64	33.5	56.8	19.0	6.24	0.67	5.36	0.05	0.01	0.08
78		45.0	15.3	8.03	34.0	56.0	19.0	6.07	0.60	5.36	0.05	0.01	0.03
79		44.6	15.1	7.86	33.9	56.7	19.2	8.10	1.16	6.56	0.14	0.03	0.11
80		44.3	14.7	8.38	33.3	52.9	17.6	4.08	0.39	3.53	0.11	0.01	0.02
Mean sd		45.4 1.89	15.3 0.67	8.31 0.332	33.8 0.36	54.7 1.36	18.5 0.51	6.73 1.197	0.78 0.284	5.70 0.955	0.11 0.045	0.01 0.007	0.09 0.038

sd Standard deviation

APPENDIX 7

(Haematology - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	LUC 10 ⁹ /l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10 ⁹ /l	TT s
3♀ 1000	61	0.04	-	-	-	-	-	-	-	-	-	1016	23
	62	0.03	-	-	-	-	-	-	-	-	-	1052	21
	63	0.04	-	-	-	-	-	-	-	-	-	999	22
	64	0.06	-	-	-	-	-	-	-	-	-	987	21
	65	0.12	-	-	-	-	-	-	-	-	-	1095	19
	66	0.07	-	-	-	-	-	-	-	-	-	984	19
	67	0.05	-	-	-	-	-	-	-	-	-	1139	20
	68	0.06	-	-	-	-	-	-	-	-	-	1068	20
	69	0.08	-	-	-	-	-	-	-	-	-	1085	19
	70	0.04	-	-	-	-	-	-	-	-	-	1008	18
	Mean	0.06										1043	20
	sd	0.026										52.6	1.3
4♀ 5000	71	0.05	-	-	-	+	-	-	-	-	-	1265	21
	72	0.04	-	-	-	-	-	-	-	-	-	1028	20
	73	0.04	-	-	-	+	-	-	-	-	-	1500	19
	74	0.06	-	-	-	-	-	-	-	-	-	1014	18
	75	0.06	-	-	-	-	-	-	-	-	-	1149	16
	76	0.06	-	-	-	-	-	-	-	-	-	1227	19
	77	0.06	-	-	-	-	-	-	-	-	-	1010	21
	78	0.02	-	-	-	-	-	-	-	-	-	1297	19
	79	0.09	-	-	-	+	-	-	-	-	-	1195	20
	80	0.02	-	-	-	-	-	-	-	-	-	1167	20
	Mean	0.05										1185	19
	sd	0.021										151.4	1.4

sd Standard deviation

APPENDIX 8

Biochemistry - individual values

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
1 δ Control	1	93	6.5	2.9	3.6	14	0.5	309	25	66	1	2.4
	2	81	6.0	3.1	2.9	15	0.5	291	25	78	<1	3.7
	3	128	6.1	2.9	3.2	14	0.7	305	38	90	1	3.7
	4	103	5.7	2.9	2.8	14	0.6	259	27	66	1	2.8
	5	127	5.8	2.9	2.9	15	0.5	270	26	100	1	2.6
	6	96	6.1	2.9	3.2	12	0.5	275	32	84	<1	1.7
	7	95	5.8	2.9	2.9	11	0.5	216	24	85	2	2.6
	8	92	6.0	2.9	3.1	12	0.5	361	31	87	<1	1.8
	9	93	6.2	2.8	3.4	14	0.5	381	27	93	1	6.2
	10	122	6.5	2.9	3.6	14	0.5	243	26	87	<1	2.2
	Mean sd	103 16.6	6.1 0.28	2.9 0.07	3.2 0.30	14 1.4	0.5 0.07	291 50.7	28 4.3	84 10.9	<1	3.0 1.32
2 δ 200	11	95	6.0	2.9	3.1	11	0.5	310	29	79	1	2.4
	12	102	5.9	3.0	2.9	12	0.5	316	29	76	1	3.7
	13	119	6.4	3.0	3.4	12	0.5	239	24	64	1	1.6
	14	112	6.1	3.0	3.1	14	0.6	225	37	90	<1	3.5
	15	108	5.9	2.9	3.0	13	0.5	415	31	69	1	2.4
	16	118	6.3	3.1	3.2	14	0.6	340	30	84	1	2.8
	17	114	6.0	2.8	3.2	12	0.5	202	24	70	1	1.7
	18	108	6.2	3.0	3.2	14	0.5	269	28	86	<1	1.4
	19	121	5.8	3.0	2.8	18	0.5	256	26	73	<1	2.0
	20	117	6.1	2.8	3.3	15	0.5	286	32	88	3	1.7
	Mean sd	111 8.2	6.1 0.19	3.0 0.10	3.1 0.18	14 2.0	0.5 0.04	286 62.5	29 3.9	78 8.9	<2	2.3 0.80

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	1	0.2	146	3.5	5.9	5.3	100	79
	2	0.2	147	4.4	5.5	5.4	104	77
	3	0.2	146	3.9	5.5	5.4	102	73
	4	0.2	147	3.7	5.6	4.9	103	66
	5	0.2	146	4.0	5.6	5.1	103	51
	6	0.2	145	3.9	5.5	4.7	103	79
	7	0.2	146	3.7	5.6	4.3	103	105
	8	0.2	145	3.8	5.6	4.9	102	68
	9	0.1	146	3.8	5.5	4.5	101	63
	10	0.2	145	4.0	5.5	4.9	103	115
	Mean sd	0.2 0.03	146 0.7	3.9 0.24	5.6 0.12	4.9 0.37	102 1.2	78 19.2
2♂ 200	11	0.2	147	3.6	5.4	4.6	104	63
	12	0.1	144	4.1	5.4	4.5	102	100
	13	0.1	145	4.1	5.5	4.1	102	91
	14	0.1	145	3.8	5.4	4.9	101	71
	15	0.2	146	4.1	5.4	5.1	103	51
	16	0.1	146	3.6	5.5	4.7	104	64
	17	0.2	145	3.7	5.6	4.1	103	97
	18	0.2	145	4.2	5.4	4.8	103	69
	19	0.2	144	4.1	5.3	4.6	102	77
	20	0.2	146	3.7	5.5	4.7	103	82
	Mean sd	0.2 0.05	145 0.9	3.9 0.24	5.4 0.08	4.6 0.32	103 0.9	77 15.9

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
3 δ 1000	21	104	6.3	3.1	3.2	13	0.6	355	30	65	<1	2.8
	22	120	6.1	3.0	3.1	14	0.6	242	27	60	1	2.3
	23	83	6.1	3.0	3.1	12	0.6	351	23	88	<1	1.9
	24	120	6.1	2.9	3.2	14	0.6	288	31	74	1	2.2
	25	116	6.0	2.9	3.1	9	0.6	301	26	67	<1	1.4
	26	111	6.1	2.7	3.4	13	0.6	234	26	63	1	3.9
	27	126	6.2	2.9	3.3	12	0.5	282	28	63	1	4.9
	28	93	6.0	3.0	3.0	14	0.6	237	20	74	<1	1.7
	29	109	6.3	3.0	3.3	13	0.5	193	23	56	1	2.4
	30	102	6.0	2.9	3.1	11	0.5	225	25	75	1	6.0
	Mean	108	6.1	2.9	3.2	13	0.6	271	26	69	<1	3.0
	sd	13.3	0.11	0.11	0.12	1.6	0.05	54.1	3.3	9.3		1.50
4 δ 5000	31	96	6.1	2.9	3.2	15	0.5	242	24	69	1	1.5
	32	104	6.0	2.9	3.1	15	0.5	238	17	56	<1	5.2
	33	113	6.4	3.1	3.3	12	0.5	310	29	72	1	4.2
	34	88	5.9	2.9	3.0	14	0.4	251	23	64	2	2.0
	35	106	6.0	2.9	3.1	11	0.5	261	22	75	1	2.2
	36	121	5.9	2.9	3.0	12	0.5	235	23	63	<1	2.9
	37	96	5.9	2.9	3.0	13	0.5	219	23	57	<1	1.3
	38	109	6.6	3.0	3.6	15	0.5	345	26	65	2	6.5
	39	102	5.5	2.8	2.7	17	0.5	208	25	57	1	2.2
	40	106	5.9	2.8	3.1	14	0.4	178	21	80	1	1.4
	Mean	104	6.0	2.9	3.1	14	0.5	249	23	66	<2	2.9
	sd	9.4	0.30	0.09	0.23	1.8	0.04	48.4	3.2	8.1		1.78

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
3♂ 1000	21	0.2	148	3.5	5.5	5.2	103	64
	22	0.2	146	3.7	5.3	4.7	103	104
	23	0.2	146	3.9	5.5	4.5	102	71
	24	0.1	146	3.9	5.6	5.0	102	57
	25	0.2	146	3.6	5.2	4.3	104	52
	26	0.2	146	3.6	5.3	5.0	104	71
	27	0.2	146	3.5	5.5	4.6	101	98
	28	0.2	147	3.5	5.5	4.8	103	82
	29	0.2	145	3.7	5.3	4.4	103	98
	30	0.2	146	3.8	5.5	4.9	103	86
	Mean	0.2	146	3.7	5.4	4.7	103	78
	sd	0.03	0.8	0.16	0.13	0.29	0.9	18.2
4♂ 5000	31	0.2	146	3.6	5.6	4.3	103	64
	32	0.2	145	3.8	5.4	4.4	103	77
	33	0.2	145	3.4	5.6	4.5	103	65
	34	0.2	146	3.6	5.5	4.9	103	111
	35	0.2	145	3.6	5.2	4.7	103	80
	36	0.1	146	3.9	5.5	4.7	106	84
	37	0.2	147	3.9	5.5	4.8	104	92
	38	0.2	145	3.6	5.4	4.3	103	55
	39	0.1	144	3.4	5.1	4.2	102	81
	40	0.2	145	4.4	5.3	4.6	103	106
	Mean	0.2	145	3.7	5.4	4.5	103	82
	sd	0.04	0.8	0.30	0.17	0.24	1.1	17.9

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
1♀ Control	41	91	6.2	3.1	3.1	16	0.5	227	25	71	<1	2.4
	42	96	6.1	3.1	3.0	16	0.5	156	25	67	1	3.5
	43	103	6.8	3.3	3.5	13	0.4	224	25	61	<1	2.2
	44	110	6.3	3.1	3.2	17	0.5	196	21	61	1	2.6
	45	106	6.1	3.0	3.1	15	0.5	191	22	59	<1	2.9
	46	108	6.3	3.1	3.2	16	0.6	204	37	75	3	3.1
	47	90	6.0	3.0	3.0	17	0.6	195	25	62	1	2.3
	48	95	6.1	3.1	3.0	13	0.4	244	28	72	1	2.0
	49	121	6.6	3.2	3.4	14	0.5	101	29	62	1	6.0
	50	99	6.2	3.3	2.9	16	0.5	119	38	70	<1	6.0
	Mean	102	6.3	3.1	3.1	15	0.5	186	28	66	<2	3.3
	sd	9.6	0.25	0.11	0.19	1.5	0.07	46.7	5.8	5.7		1.49
2♀ 200	51	90	6.7	3.3	3.4	15	0.5	145	51	100	1	6.8
	52	101	6.5	3.2	3.3	17	0.5	117	34	75	<1	7.4
	53	115	6.3	3.2	3.1	16	0.5	188	29	74	5	1.5
	54	96	6.4	3.1	3.3	16	0.6	167	30	76	<1	2.9
	55	112	6.4	3.5	2.9	13	0.6	164	23	53	1	2.7
	56	110	6.4	3.1	3.3	15	0.5	180	34	69	2	6.2
	57	98	6.8	3.4	3.4	14	0.3	182	21	69	1	3.1
	58	95	6.8	3.1	3.7	18	0.6	226	21	75	<1	2.0
	59	100	6.7	3.3	3.4	18	0.6	165	34	83	1	4.9
	60	102	6.0	3.2	2.8	16	0.5	165	23	65	1	2.7
	Mean	102	6.5	3.2	3.3	16	0.5	170	30	74	<2	4.0
	sd	8.0	0.25	0.13	0.26	1.6	0.09	28.3	9.1	12.2		2.13

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1 ♀ Control	41	0.1	145	3.5	5.4	4.0	105	84
	42	0.3	144	4.0	5.5	4.1	106	89
	43	0.2	145	3.4	5.7	3.9	105	88
	44	0.2	144	3.9	5.6	3.6	105	103
	45	0.2	144	3.8	5.5	4.0	105	94
	46	0.2	144	3.3	5.4	4.5	102	115
	47	0.2	146	5.7	5.2	4.8	107	88
	48	0.2	145	3.4	5.5	3.9	103	60
	49	0.2	143	3.8	5.4	3.9	103	85
	50	0.2	145	3.5	5.4	3.7	105	93
	Mean sd	0.2 0.05	145 0.8	3.8 0.70	5.5 0.13	4.0 0.36	105 1.5	90 14.1
2 ♀ 200	51	0.2	144	3.8	5.8	4.4	103	81
	52	0.2	141	4.1	5.3	3.7	103	87
	53	0.1	145	3.4	5.4	3.1	106	77
	54	0.2	143	3.6	5.3	3.6	106	95
	55	0.2	146	3.1	5.4	4.2	104	76
	56	0.2	147	3.5	5.4	3.6	108	125
	57	0.2	146	3.5	5.7	3.8	104	101
	58	0.2	144	3.8	5.4	3.9	105	89
	59	0.2	145	3.4	5.4	3.8	105	84
	60	0.2	145	3.6	5.4	3.9	106	100
	Mean sd	0.2 0.03	145 1.7	3.6 0.27	5.5 0.16	3.8 0.35	105 1.6	92 14.7

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
3♀ 1000	61	119	6.8	3.4	3.4	12	0.5	143	20	50	1	4.1
	62	88	6.2	3.2	3.0	15	0.6	117	23	65	1	1.3
	63	111	6.4	3.1	3.3	14	0.6	212	24	60	1	1.3
	64	109	6.3	3.1	3.2	15	0.5	174	31	71	<1	1.7
	65	97	6.6	3.5	3.1	16	0.5	95	41	95	1	3.8
	66	109	6.4	3.2	3.2	17	0.5	205	24	60	1	2.6
	67	95	6.1	3.1	3.0	17	0.6	193	20	64	1	1.5
	68	119	6.1	3.2	2.9	15	0.6	190	25	63	<1	2.7
	69	101	6.6	3.1	3.5	14	0.4	226	22	57	1	2.9
	70	117	6.3	3.0	3.3	15	0.5	196	23	65	<1	2.5
	Mean	107	6.4	3.2	3.2	15	0.5	175	25	65	<1	2.4
	sd	10.8	0.23	0.15	0.19	1.5	0.07	43.0	6.3	11.9		1.00
4♀ 5000	71	100	6.7	3.3	3.4	15	0.4	200	20	58	<1	1.9
	72	90	6.2	3.1	3.1	16	0.5	124	16	51	1	1.6
	73	103	6.1	3.2	2.9	15	0.5	161	15	51	1	2.2
	74	104	6.1	3.3	2.8	11	0.5	132	16	49	1	3.1
	75	110	6.0	3.1	2.9	12	0.4	161	17	54	1	2.4
	76	120	6.9	3.7	3.2	15	0.4	138	24	65	<1	2.2
	77	110	6.6	3.3	3.3	13	0.5	161	16	48	<1	2.9
	78	115	6.7	3.4	3.3	12	0.5	147	17	52	5	3.0
	79	93	6.3	3.3	3.0	11	0.4	151	19	58	<1	1.5
	80	109	6.8	3.5	3.3	13	0.4	120	17	48	<1	1.7
	Mean	105	6.4	3.3	3.1	13	0.5	150	18	53	<2	2.3
	sd	9.4	0.33	0.18	0.21	1.8	0.05	23.4	2.7	5.5		0.59

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 5 (9 July 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
3♀ 1000	61	0.2	144	3.5	5.6	4.1	102	85
	62	0.2	146	3.8	5.4	4.4	106	74
	63	0.2	145	3.3	5.4	3.7	106	76
	64	0.2	144	3.4	5.4	4.0	104	83
	65	0.2	143	4.4	5.5	4.1	104	78
	66	0.2	144	3.6	5.3	3.6	105	71
	67	0.2	143	3.5	5.2	3.9	105	64
	68	0.2	145	3.9	5.3	3.6	106	65
	69	0.2	145	3.3	5.5	4.1	104	69
	70	0.2	144	3.3	5.3	3.0	104	107
	Mean sd	0.2 0.00	144 0.9	3.6 0.35	5.4 0.12	3.9 0.39	105 1.3	77 12.6
4♀ 5000	71	0.2	143	3.5	5.4	3.8	102	77
	72	0.2	146	3.4	5.4	4.2	106	94
	73	0.2	145	3.6	5.3	3.5	105	78
	74	0.2	144	3.7	5.5	3.9	104	76
	75	0.2	146	3.3	5.4	3.8	105	99
	76	0.3	144	3.3	5.7	3.7	101	101
	77	0.2	145	3.4	5.4	3.5	105	116
	78	0.2	145	3.7	5.3	3.7	106	91
	79	0.2	144	3.6	5.5	4.3	104	91
	80	0.2	143	3.7	5.5	3.8	102	102
	Mean sd	0.2 0.03	145 1.1	3.5 0.16	5.4 0.12	3.8 0.26	104 1.8	93 12.9

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
1 σ Control	1	122	6.6	2.6	4.0	13	0.5	189	26	57	<1	3.4
	2	114	6.8	3.1	3.7	15	0.6	223	29	66	1	4.9
	3	101	6.9	2.9	4.0	14	0.5	195	43	68	<1	4.3
	4	103	6.5	2.9	3.6	14	0.5	161	24	58	<1	4.0
	5	133	6.8	2.9	3.9	15	0.5	214	48	80	<1	7.6
	6	107	6.5	3.0	3.5	12	0.4	167	32	57	1	5.8
	7	129	6.3	2.9	3.4	13	0.5	148	29	58	<1	3.3
	8	116	6.9	2.9	4.0	12	0.5	224	35	62	2	2.1
	9	121	6.2	2.6	3.6	15	0.6	228	29	61	<1	8.6
	10	119	7.2	2.9	4.3	12	0.4	176	26	52	1	4.1
	Mean sd	117 10.6	6.7 0.31	2.9 0.16	3.8 0.28	14 1.3	0.5 0.07	193 29.0	32 7.8	62 7.9	<1	4.8 2.00
2 σ 200	11	113	6.3	2.6	3.7	11	0.5	171	27	56	<1	4.0
	12	113	6.7	2.8	3.9	13	0.6	200	30	59	1	1.9
	13	132	6.3	2.7	3.6	11	0.4	167	27	48	1	5.3
	14	118	6.6	2.8	3.8	15	0.4	211	43	58	1	4.4
	15	125	6.7	2.8	3.9	12	0.5	278	32	52	<1	2.8
	16	118	6.7	3.0	3.7	12	0.5	206	41	83	1	4.2
	17	135	6.4	2.9	3.5	12	0.5	138	31	58	<1	3.0
	18	112	6.2	2.8	3.4	17	0.4	182	26	52	<1	1.3
	19	123	6.5	2.9	3.6	16	0.6	177	30	50	<1	6.1
	20	126	6.8	2.7	4.1	14	0.5	165	33	62	<1	6.9
	Mean sd	122 8.1	6.5 0.21	2.8 0.12	3.7 0.21	13 2.1	0.5 0.07	190 38.0	32 5.8	58 9.9	<1	4.0 1.79

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	1	0.2	146	3.9	5.7	4.5	101	75
	2	0.2	145	4.0	5.6	3.8	102	68
	3	0.2	145	3.7	5.6	3.7	103	72
	4	0.3	145	3.9	5.5	3.8	103	70
	5	0.2	145	4.0	5.7	4.5	102	60
	6	0.2	145	3.7	5.5	3.5	102	93
	7	0.3	144	3.7	5.7	3.9	101	108
	8	0.2	144	3.7	5.7	3.8	100	78
	9	0.3	145	3.6	5.6	4.0	101	64
	10	0.2	144	3.7	5.8	3.7	101	137
	Mean sd	0.2 0.05	145 0.6	3.8 0.14	5.6 0.10	3.9 0.33	102 1.0	83 23.9
2♂ 200	11	0.2	146	3.8	5.3	3.5	104	60
	12	0.2	145	4.0	5.5	3.8	103	84
	13	0.2	144	3.9	5.6	3.6	102	87
	14	0.2	144	4.1	5.6	3.8	101	91
	15	0.4	144	3.8	5.5	3.6	101	47
	16	0.2	145	4.0	5.3	3.8	105	64
	17	0.2	145	3.7	5.5	3.7	102	82
	18	0.1	145	4.0	5.3	3.9	102	71
	19	0.2	145	3.5	5.6	3.9	99	78
	20	0.2	145	3.8	5.5	3.5	102	101
	Mean sd	0.2 0.07	145 0.6	3.9 0.18	5.5 0.13	3.7 0.15	102 1.7	77 16.1

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ .ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
3♂ 1000	21	100	6.5	2.7	3.8	14	0.4	207	38	74	<1	1.8
	22	113	6.8	2.8	4.0	13	0.5	126	39	59	1	3.6
	23	98	6.8	2.7	4.1	12	0.5	154	24	64	1	2.7
	24	117	6.6	2.7	3.9	13	0.5	149	37	59	1	6.4
	25	139	6.3	2.7	3.6	10	0.4	173	35	57	<1	3.5
	26	105	6.4	2.5	3.9	13	0.4	126	31	54	2	5.0
	27	136	6.8	2.6	4.2	13	0.5	159	32	56	3	4.1
	28	97	6.8	3.0	3.8	16	0.5	148	18	54	<1	2.5
	29	119	6.6	2.9	3.7	15	0.4	144	28	63	<1	5.0
	30	114	6.9	3.0	3.9	16	0.4	178	32	50	<1	2.6
	Mean	114	6.7	2.8	3.9	14	0.5	156	31	59	<2	3.7
	sd	14.8	0.20	0.16	0.18	1.8	0.05	24.6	6.6	6.7		1.42
4♂ 5000	31	113	6.6	2.8	3.8	13	0.5	136	24	50	<1	4.1
	32	119	6.5	2.8	3.7	13	0.4	139	18	41	<1	3.4
	33	131	6.6	2.7	3.9	12	0.5	180	26	53	<1	4.2
	34	96	6.2	2.7	3.5	12	0.4	153	26	54	3	8.6
	35	112	6.4	2.6	3.8	13	0.4	163	27	48	1	3.0
	36	130	6.3	2.5	3.8	16	0.5	144	24	53	<1	5.1
	37	119	6.8	2.8	4.0	12	0.5	134	30	66	2	3.0
	38	121	6.6	2.7	3.9	17	0.6	237	34	62	<1	2.6
	39	105	6.1	2.6	3.5	14	0.5	127	30	62	<1	7.2
	40	125	6.8	2.9	3.9	15	0.5	130	24	46	1	2.3
	Mean	117	6.5	2.7	3.8	14	0.5	154	26	54	<2	4.4
	sd	10.9	0.24	0.12	0.17	1.8	0.06	33.3	4.4	7.9		2.07

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
3♂ 1000	21	0.3	146	3.9	5.4	4.0	104	65
	22	0.2	144	4.0	5.4	3.7	101	120
	23	0.1	145	3.6	5.5	3.4	102	64
	24	0.1	145	3.5	5.5	3.5	101	73
	25	0.2	145	3.9	5.3	3.8	102	52
	26	0.2	146	4.5	5.5	4.8	103	82
	27	0.2	145	3.4	5.6	3.8	101	100
	28	0.2	146	3.4	5.5	4.1	100	84
	29	0.2	143	5.1	5.2	3.9	102	111
	30	0.2	145	3.6	5.6	3.6	102	99
	Mean	0.2	145	3.9	5.5	3.9	102	85
	sd	0.06	0.9	0.54	0.13	0.39	1.1	22.1
4♂ 5000	31	0.2	147	3.5	5.4	3.6	104	66
	32	0.2	144	4.2	5.4	3.5	102	82
	33	0.2	145	3.5	5.4	3.3	102	69
	34	0.2	146	3.6	5.3	3.9	102	101
	35	0.2	143	3.7	5.3	3.7	101	81
	36	0.2	146	3.6	5.4	3.9	103	83
	37	0.2	144	3.7	5.6	3.8	102	100
	38	0.2	144	3.7	5.4	3.3	102	75
	39	0.2	144	3.8	5.2	3.8	103	80
	40	0.2	145	3.6	5.4	3.4	101	110
	Mean	0.2	145	3.7	5.4	3.6	102	85
	sd	0.00	1.2	0.20	0.10	0.23	0.9	14.4

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
1♀ Control	41	102	6.8	3.2	3.6	15	0.5	145	27	61	1	3.5
	42	118	6.6	3.3	3.3	18	0.6	118	32	56	<1	5.3
	43	107	6.9	3.4	3.5	13	0.5	112	27	50	<1	2.2
	44	95	6.5	3.1	3.4	16	0.5	85	18	45	<1	4.3
	45	114	6.2	2.8	3.4	14	0.5	104	32	62	<1	6.7
	46	96	6.5	2.9	3.6	13	0.5	118	34	66	<1	4.5
	48	112	6.2	2.9	3.3	12	0.4	124	24	59	<1	2.8
	49	175	6.7	3.1	3.6	15	0.5	75	211	318	<1	3.1
	50	130	6.8	3.3	3.5	13	0.5	71	39	65	<1	3.0
	Mean	117	6.6	3.1	3.5	14	0.5	106	49	87	<1	3.9
	sd	24.5	0.25	0.21	0.12	1.9	0.05	24.5	60.9	86.9		1.42
2♀ 200	51	118	7.0	3.3	3.7	16	0.5	125	33	64	1	5.9
	52	105	7.2	3.1	4.1	14	0.5	75	32	63	1	11.4
	53	124	7.2	3.5	3.7	16	0.6	140	115	129	<1	14.7
	54	105	6.6	3.2	3.4	16	0.5	80	51	86	<1	3.0
	55	120	7.2	3.7	3.5	15	0.5	78	28	47	<1	2.6
	56	132	6.8	3.1	3.7	15	0.5	94	72	120	<1	2.5
	57	123	7.2	3.5	3.7	14	0.5	71	49	69	2	3.6
	58	126	7.7	3.3	4.4	13	0.5	88	32	59	1	2.9
	59	141	6.8	3.2	3.6	15	0.7	77	29	67	<1	5.1
	60	138	6.7	3.3	3.4	16	0.6	81	24	60	<1	1.5
	Mean	123	7.0	3.3	3.7	15	0.5	91	47	76	<1	5.3
	sd	12.1	0.33	0.19	0.31	1.1	0.07	23.1	28.1	27.2		4.34

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♀ Control	41	0.2	145	3.2	5.3	2.9	104	104
	42	0.3	144	4.8	5.5	3.5	104	99
	43	0.2	145	3.8	5.4	3.1	103	90
	44	0.2	144	3.4	5.4	3.1	104	115
	45	0.2	143	4.5	5.3	3.1	104	112
	46	0.2	144	3.8	5.6	3.9	103	105
	48	0.2	144	3.6	5.6	3.5	102	74
	49	0.3	142	5.9	5.5	4.4	101	80
	50	0.2	144	3.2	5.4	2.7	103	90
	Mean	0.2	144	4.0	5.4	3.4	103	97
	sd	0.04	0.9	0.89	0.11	0.53	1.1	14.1
2♀ 200	51	0.2	145	3.2	5.6	3.3	103	74
	52	0.3	143	3.8	5.6	3.0	103	105
	53	0.3	144	3.1	5.6	2.5	102	106
	54	0.3	145	3.7	5.4	3.1	105	96
	55	0.3	144	3.3	5.5	2.8	103	74
	56	0.1	143	4.0	5.5	3.2	103	148
	57	0.3	145	3.3	5.8	3.1	101	117
	58	0.3	142	3.4	5.7	2.6	101	107
	59	0.3	143	2.8	5.4	2.6	102	97
	60	0.2	145	3.4	5.3	2.7	105	96
	Mean	0.3	144	3.4	5.5	2.9	103	102
	sd	0.07	1.1	0.35	0.15	0.28	1.4	21.2

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	γ GT mU/ ml	OCT mU/ ml
			Total	Alb	Glob							
3♀ 1000	61	138	6.9	3.2	3.7	11	0.5	77	26	43	<1	3.3
	62	131	6.6	3.2	3.4	14	0.6	68	26	58	3	4.1
	63	122	6.9	3.0	3.9	15	0.6	112	25	47	1	3.2
	64	117	6.5	2.8	3.7	18	0.6	81	23	51	<1	7.4
	65	127	7.6	3.9	3.7	18	0.5	52	33	52	<1	2.5
	66	128	6.7	3.3	3.4	18	0.5	164	29	60	<1	2.4
	67	124	6.4	3.1	3.3	16	0.6	86	23	49	<1	2.8
	68	128	6.6	3.2	3.4	15	0.5	118	24	49	<1	3.2
	69	116	6.8	3.2	3.6	14	0.5	116	23	67	<1	3.0
	70	113	6.6	3.0	3.6	14	0.6	79	31	80	<1	7.4
	Mean	124	6.8	3.2	3.6	15	0.6	95	26	56	<1	3.9
	sd	7.6	0.34	0.29	0.19	2.3	0.05	32.4	3.6	11.1		1.89
4♀ 5000	71	112	6.5	2.8	3.7	16	0.5	162	24	52	<1	7.8
	72	116	6.4	3.1	3.3	16	0.5	89	23	57	<1	1.5
	73	105	6.3	2.9	3.4	17	0.6	102	27	53	1	16.3
	74	116	7.7	3.6	4.1	13	0.6	50	17	42	<1	7.4
	75	124	7.5	3.6	3.9	14	0.4	77	70	108	<1	5.1
	76	124	7.3	3.6	3.7	10	0.4	60	47	64	<1	6.3
	77	127	7.3	3.5	3.8	13	0.5	70	16	41	<1	13.1
	78	136	7.3	3.6	3.7	15	0.6	94	15	39	<1	3.5
	79	122	7.0	3.6	3.4	13	0.5	80	21	48	<1	4.1
	80	131	6.7	3.5	3.2	14	0.5	70	14	46	<1	1.9
	Mean	121	7.0	3.4	3.6	14	0.5	85	27	55	<1	6.7
	sd	9.2	0.49	0.32	0.29	2.0	0.07	31.1	17.8	20.2		4.77

sd Standard deviation

APPENDIX 8

(Biochemistry - continued)

Week 13 (6 September 1996)

Group/ dosage/ ppm	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
3♀ 1000	61	0.3	144	3.7	5.6	2.7	102	87
	62	0.2	145	3.5	5.2	2.8	103	77
	63	0.3	144	3.5	5.6	2.5	103	83
	64	0.2	143	3.4	5.3	3.0	104	88
	65	0.3	142	3.2	5.7	3.1	100	104
	66	0.3	144	3.3	5.6	3.1	104	96
	67	0.2	143	3.4	5.2	2.5	103	91
	68	0.2	144	3.4	5.4	2.5	104	72
	69	0.2	143	3.4	5.5	2.7	101	85
	70	0.3	144	4.1	5.5	2.9	103	122
	Mean	0.3	144	3.5	5.5	2.8	103	91
	sd	0.05	0.8	0.25	0.18	0.24	1.3	14.3
4♀ 5000	71	0.2	142	3.3	5.1	2.5	101	79
	72	0.2	144	3.3	5.3	2.3	104	85
	73	0.2	144	3.4	5.4	3.1	102	99
	74	0.3	144	3.0	5.9	2.4	100	113
	75	0.3	144	3.1	5.7	2.6	101	124
	76	0.3	143	3.6	5.8	2.7	101	105
	77	0.2	145	3.5	5.6	2.2	104	121
	78	0.2	142	3.3	5.4	2.3	102	94
	79	0.3	143	3.6	5.6	2.6	102	99
	80	0.1	143	3.6	5.2	2.3	103	85
	Mean	0.2	143	3.4	5.5	2.5	102	100
	sd	0.07	1.0	0.21	0.26	0.27	1.3	15.4

sd Standard deviation

APPENDIX 9

Organ weights - individual values

Week 14

Group/ dosage/ ppm	Animal no.	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g
1♂ Control	1	615	2.04	13.6	28.9	1.82	20.7	0.95	3.69
	2	522	2.26	15.4	23.9	1.45	19.1	0.90	3.58
	3	497	2.13	14.5	17.8	1.42	17.7	0.51	3.23
	4	567	2.17	13.3	29.5	1.74	23.6	1.14	4.17
	5	568	2.04	16.4	24.2	1.74	24.3	0.85	4.09
	6	561	2.04	13.4	22.3	1.77	20.5	0.91	3.67
	7	543	2.03	16.8	27.3	1.64	21.9	0.88	3.77
	8	579	2.20	14.4	19.3	1.72	22.9	0.99	4.46
	9	575	2.16	19.0	25.3	1.89	19.4	1.14	3.90
	10	555	1.78	13.8	19.0	1.86	23.6	1.02	4.03
	Mean sd	558 32.3	2.08 0.133	15.1 1.85	23.8 4.15	1.71 0.158	21.4 2.23	0.93 0.177	3.86 0.349
2♂ 200	11	560	2.03	13.2	20.0	1.77	25.9	0.85	3.90
	12	574	2.14	15.7	24.8	1.81	22.4	1.02	3.34
	13	605	1.99	15.4	24.2	1.79	27.1	0.96	4.06
	14	668	2.02	14.3	25.9	1.65	26.6	0.99	3.59
	15	553	2.31	13.6	23.6	1.79	23.6	0.85	4.24
	16	552	2.15	14.4	15.2	1.66	17.8	0.81	3.61
	17	614	2.10	15.5	25.5	1.67	22.2	0.91	4.34
	18	459	2.09	11.1	27.9	1.38	18.9	0.94	3.43
	19	510	2.08	14.2	15.9	1.69	21.9	0.88	3.35
	20	581	2.15	22.4	20.9	1.80	25.9	0.76	3.55
	Mean sd	568 57.2	2.11 0.091	15.0 2.94	22.4 4.28	1.70 0.128	23.2 3.20	0.90 0.085	3.74 0.369

sd Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Animal no.	Adrenals	Prostate	Testes		Seminal	Epididymides	
		mg	g	Left g	Right g	Vesicle g	Left g	Right g
1♂ Control	1	55.9	1.445	1.779	1.788	1.51	0.768	0.841
	2	64.2	1.033	1.565	1.613	2.35	0.680	0.699
	3	54.9	0.989	1.677	1.658	1.40	0.584	0.633
	4	70.4	1.703	1.842	1.827	1.56	0.716	0.819
	5	56.2	0.927	1.526	1.555	1.74	0.631	0.637
	6	65.1	1.367	1.818	1.856	1.32	0.630	0.693
	7	73.1	1.066	1.656	1.618	1.44	0.620	0.586
	8	54.5	1.488	1.758	1.823	1.61	0.709	0.765
	9	54.9	1.082	1.655	1.633	1.16	0.644	0.642
	10	60.6	1.343	1.849	1.899	1.50	0.702	0.756
	Mean	61.0	1.244	1.713	1.727	1.56	0.668	0.707
	sd	6.89	0.2590	0.1142	0.1235	0.320	0.0558	0.0855
2♂ 200	11	57.2	1.516	1.981	2.061	1.05	0.697	0.734
	12	44.0	0.930	1.702	1.700	1.24	0.599	0.646
	13	49.6	1.223	1.864	1.813	1.82	0.733	0.762
	14	60.6	0.952	1.593	1.568	1.18	0.630	0.673
	15	66.7	1.485	1.844	1.811	1.12	0.703	0.712
	16	53.1	1.136	1.888	1.915	1.38	0.737	0.753
	17	57.9	2.037	1.893	1.906	1.41	0.643	0.664
	18	53.9	0.885	1.748	1.870	1.61	0.694	0.755
	19	63.8	1.203	1.505	1.632	1.59	0.601	0.668
	20	82.0	0.584	1.882	1.764	1.38	0.672	0.666
	Mean	58.9	1.195	1.790	1.804	1.38	0.671	0.703
	sd	10.50	0.4081	0.1502	0.1457	0.242	0.0505	0.0447

sd Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Animal no.	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g
3♂ 1000	21	520	2.09	10.1	13.8	1.66	18.9	1.00	3.52
	22	567	2.04	13.4	24.7	1.90	26.4	1.08	4.66
	23	535	2.25	16.0	21.9	1.48	19.3	0.88	3.89
	24	587	2.12	16.9	24.2	1.71	27.7	1.09	4.18
	25	584	1.91	14.8	25.3	1.64	27.1	0.82	4.25
	26	516	2.02	13.9	20.3	1.57	19.8	0.76	3.32
	27	573	2.10	15.8	21.9	1.61	18.7	0.94	4.01
	28	524	1.99	11.3	22.9	1.65	24.2	0.63	3.75
	29	482	1.98	12.4	17.8	1.66	16.9	0.82	4.08
	30	521	2.13	17.6	21.4	1.78	22.6	0.65	3.50
	Mean	541	2.06	14.2	21.4	1.67	22.2	0.87	3.92
	sd	35.0	0.098	2.45	3.47	0.114	3.98	0.163	0.407
4♂ 5000	31	482	2.05	15.7	22.2	1.57	22.3	0.68	3.27
	32	435	2.09	10.8	22.7	1.27	16.9	0.77	3.09
	33	540	2.12	14.2	23.6	1.59	25.2	0.86	3.65
	34	466	1.99	16.1	24.0	1.56	19.2	0.85	3.34
	35	487	2.01	15.4	23.1	1.63	21.9	0.69	3.21
	36	509	2.05	14.2	25.1	1.73	24.3	0.89	4.06
	37	442	1.99	11.4	21.3	1.39	15.9	0.70	2.78
	38	456	2.00	16.7	16.1	1.54	19.7	0.74	2.69
	39	476	2.09	12.4	19.7	1.84	17.2	0.88	3.31
	40	559	2.22	17.8	25.5	1.85	27.8	0.81	4.31
	Mean	485	2.06	14.5	22.3	1.60	21.1	0.79	3.37
	sd	40.4	0.072	2.32	2.78	0.182	3.95	0.082	0.512

sd Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Animal no.	Adrenals	Prostate	Testes		Seminal	Epididymides	
		mg	g	Left g	Right g	Vesicle g	Left g	Right g
3♂ 1000	21	55.9	1.031	1.986	2.038	1.39	0.675	0.763
	22	72.5	1.514	1.768	1.765	1.40	0.676	0.718
	23	52.6	0.870	1.712	1.727	1.07	0.668	0.698
	24	74.0	1.134	1.449	1.518	1.89	0.637	0.698
	25	54.5	1.426	1.664	1.643	1.58	0.623	0.726
	26	50.4	1.587	1.658	1.678	0.96	0.637	0.672
	27	58.3	1.026	1.765	1.826	1.71	0.647	0.710
	28	75.3	1.020	1.762	1.771	2.08	0.716	0.681
	29	51.4	1.322	1.581	1.598	1.54	0.540	0.578
	30	72.7	0.995	1.808	1.800	0.99	0.678	0.621
	Mean	61.8	1.193	1.715	1.736	1.46	0.650	0.687
	sd	10.47	0.2495	0.1431	0.1430	0.376	0.0471	0.0533
4♂ 5000	31	51.3	1.086	1.686	1.681	1.48	0.659	0.694
	32	69.0	1.214	1.583	1.611	0.97	0.639	0.673
	33	63.8	0.724	1.515	1.448	0.97	0.639	0.691
	34	69.3	1.032	1.807	1.832	1.33	0.740	0.725
	35	55.9	1.284	1.696	1.759	1.33	0.687	0.711
	36	54.9	1.172	1.666	1.649	1.59	0.602	0.685
	37	54.3	1.107	1.938	2.043	1.30	0.718	0.759
	38	42.5	0.735	1.563	1.558	1.09	0.585	0.598
	39	50.8	1.305	1.773	1.752	1.24	0.616	0.638
	40	66.4	1.600	1.809	1.709	1.91	0.672	0.667
	Mean	57.8	1.126	1.704	1.704	1.32	0.656	0.684
	sd	8.93	0.2618	0.1305	0.1618	0.289	0.0497	0.0449

sd Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Animal no.	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g	Adrenals mg	Uterus g	Ovaries mg
1 ♀ Control	41	262	2.02	13.9	13.8	0.87	9.8	0.54	1.89	72.2	0.68	64.2
	42	279	1.87	14.1	16.8	0.93	10.5	0.69	2.17	83.8	0.44	77.7
	43	310	1.98	16.8	18.2	1.04	13.2	0.61	2.31	66.9	0.58	109.1
	44	307	1.95	12.4	17.6	1.01	12.8	0.63	2.22	73.1	0.58	85.1
	45	276	1.87	16.4	17.6	0.93	12.1	0.61	2.32	75.3	0.50	82.2
	46	279	1.92	15.2	17.3	1.13	10.1	0.44	1.95	83.7	0.52	68.3
	48	255	1.91	19.5	19.8	1.09	10.4	0.61	2.46	73.7	0.59	86.3
	49	237	1.65	16.8	14.8	0.90	10.7	0.45	2.05	59.8	0.45	76.1
	50	235	1.81	11.9	13.1	0.90	10.4	0.51	1.81	63.1	0.69	83.8
	Mean	271	1.89	15.2	16.6	0.98	11.1	0.56	2.13	72.4	0.56	81.4
	sd	26.8	0.107	2.42	2.20	0.092	1.23	0.084	0.220	8.24	0.090	12.85
2 ♀ 200	51	257	1.81	11.0	16.4	1.22	9.2	0.68	2.04	62.0	1.08	72.5
	52	252	1.79	11.7	15.5	1.08	8.3	0.38	1.85	57.3	0.82	88.0
	53	304	1.95	19.3	18.1	1.04	12.2	0.67	2.20	82.4	0.76	74.4
	54	294	1.89	16.6	16.8	0.99	11.3	0.55	1.97	71.6	0.49	97.3
	55	273	1.75	16.6	11.3	0.90	10.4	0.49	2.26	69.1	0.51	70.0
	56	339	1.88	17.1	18.3	0.97	11.7	0.73	2.09	62.8	0.66	82.9
	57	271	1.75	13.2	14.6	1.03	10.7	0.47	2.21	56.9	0.57	70.0
	58	300	1.71	25.7	20.5	1.25	12.6	0.58	2.02	87.6	0.68	74.5
	59	257	1.83	14.3	19.2	0.91	11.0	0.50	1.81	62.7	0.65	78.0
	60	279	1.87	16.1	15.1	0.92	12.0	0.55	2.05	68.5	0.98	87.3
	Mean	282	1.82	16.2	16.6	1.03	10.9	0.56	2.05	68.1	0.72	79.5
	sd	27.0	0.074	4.23	2.64	0.121	1.36	0.109	0.151	10.19	0.195	9.09

sd Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 14

Group/ dosage/ ppm	Animal no.	Body wt g	Brain g	Pitu- itary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g	Adrenals mg	Uterus g	Ovaries mg
3♀ 1000	61	377	1.91	14.0	19.0	1.22	14.1	0.64	2.56	60.3	0.90	105.0
	62	265	1.87	12.8	13.5	0.92	9.9	0.43	1.94	69.0	1.10	72.5
	63	286	1.96	18.2	16.6	1.05	11.6	0.60	2.06	82.5	1.14	77.0
	64	290	1.94	15.3	16.5	1.09	12.8	0.69	2.44	79.6	0.63	88.2
	65	282	1.77	14.5	13.3	1.04	12.5	0.48	2.61	65.6	1.13	62.4
	66	255	1.95	15.2	14.6	1.03	10.4	0.56	2.81	69.9	0.52	81.5
	67	308	1.93	18.3	17.3	1.09	11.3	0.73	2.15	102.9	1.12	89.3
	68	221	1.62	8.1	13.9	0.93	9.2	0.55	2.12	55.6	0.47	66.0
	69	251	2.01	19.5	15.6	1.13	9.8	0.43	2.33	77.1	0.58	66.6
	70	271	1.80	16.0	20.3	1.03	12.7	0.77	2.28	54.8	0.56	77.2
	Mean	281	1.88	15.2	16.1	1.05	11.4	0.59	2.33	71.7	0.81	78.6
	sd	41.4	0.116	3.26	2.35	0.087	1.58	0.120	0.273	14.55	0.289	12.99
4♀ 5000	71	261	1.79	17.7	16.2	1.01	10.8	0.59	2.21	63.2	0.85	107.2
	72	232	1.85	14.6	17.5	0.86	8.1	0.56	1.86	54.5	0.56	86.1
	73	261	1.86	16.2	15.1	1.04	10.6	0.45	2.13	81.3	0.46	75.9
	74	279	1.85	20.0	19.6	1.11	12.3	0.48	2.66	79.9	0.76	80.0
	75	301	1.90	23.1	21.8	1.15	13.7	0.59	3.05	65.9	0.99	52.6
	76	274	1.91	16.0	18.2	1.03	12.1	0.52	2.52	62.4	1.42	94.4
	77	287	1.85	13.8	25.8	1.18	13.3	0.56	2.17	59.4	1.05	92.3
	78	263	1.85	13.4	16.3	0.94	12.3	0.52	2.08	57.4	0.99	90.8
	79	275	1.85	20.6	13.7	1.02	14.7	0.54	2.25	65.2	1.04	86.2
	80	240	1.80	18.7	15.8	0.92	9.6	0.48	2.06	53.7	0.57	127.6
	Mean	267	1.85	17.4	18.0	1.03	11.7	0.53	2.30	64.3	0.87	89.3
	sd	20.7	0.036	3.19	3.59	0.101	1.99	0.047	0.349	9.55	0.292	19.60

sd Standard deviation

APPENDIX 10

Brain measurements

Group/ dosage/ ppm	Animal no.	Bodyweight (g)	Brain parameters		
			Weight (g)	Length (mm)	Width (mm)
1♂ Control	81	570	1.72	21.90	14.52
	82	512	1.66	20.05	14.78
	83	532	1.69	21.02	14.43
	84	515	1.70	20.47	15.14
	85	518	1.71	21.37	14.62
	Mean sd	529 24.1	1.70 0.025	21.0 0.73	14.7 0.28
2♂ 200	86	448	1.57	20.74	14.31
	87	605	1.70	20.95	14.76
	88	557	1.79	20.81	15.29
	89	586	1.79	20.39	13.85
	90	574	1.72	21.83	14.78
	Mean sd	553 62.0	1.71 0.088	20.9 0.54	14.6 0.54
3♂ 1000	91	506	1.83	21.22	15.32
	92	580	1.71	20.89	14.17
	93	574	1.78	20.98	14.89
	94	558	1.64	20.84	14.89
	95	555	1.57	20.30	14.70
	Mean sd	555 29.2	1.71 0.102	20.8 0.34	14.8 0.42
4♂ 5000	96	486	1.74	21.59	14.68
	97	452	1.71	21.46	14.35
	98	542	1.90	22.15	14.56
	99	500	1.61	21.53	14.53
	100	407	1.56	20.70	14.46
	Mean sd	477 50.9	1.70 0.135	21.5 0.52	14.5 0.12

sd Standard deviation

APPENDIX 10

(Brain measurements - continued)

Group/ dosage/ ppm	Animal no.	Bodyweight (g)	Brain parameters		
			Weight (g)	Length (mm)	Width (mm)
1 ♀ Control	101	296	1.65	20.56	13.77
	102	321	1.59	20.45	14.63
	103	351	1.55	19.60	14.01
	104	283	1.60	20.57	14.48
	105	320	1.63	19.97	14.20
	Mean sd	314 26.4	1.60 0.038	20.2 0.43	14.2 0.35
2 ♀ 200	106	245	1.47	20.22	13.20
	107	311	1.48	21.12	14.64
	108	360	1.62	20.87	14.35
	109	326	1.61	20.51	13.93
	110	304	1.45	20.27	14.66
	Mean sd	309 41.7	1.53 0.081	20.6 0.39	14.2 0.61
3 ♀ 1000	111	333	1.61	20.02	13.16
	112	264	1.43	19.60	13.41
	113	351	1.54	19.96	14.00
	114	243	1.58	19.53	14.02
	115	271	1.50	20.64	14.49
	Mean sd	293 46.9	1.53 0.069	20.0 0.44	13.8 0.53
4 ♀ 5000	116	223	1.34	19.44	13.05
	117	268	1.68	21.24	14.21
	118	317	1.58	19.21	14.91
	119	284	1.60	20.25	14.38
	120	299	1.62	21.33	14.49
	Mean sd	278 35.9	1.57 0.131	20.3 0.98	14.2 0.70

sd Standard deviation

APPENDIX 11

Individual clinical and pathological findings

In this appendix the clinical, macroscopic and microscopic findings relating to each animal are listed.

The initial examination was undertaken by the study pathologist, the results of which were then subjected to a routine peer review by a second pathologist. The diagnoses reported here represent the consensus opinions of both pathologists.

Study Pathologist: Susan Harling, B.V.M.S., M.R.C.V.S.,
Senior Pathologist
Department of Pathology

Peer Review: Dianne Creasy, C.I.Biol., M.I.Biol., Ph.D., Dip.R.C.Path.(Tox.), M.R.C.Path.,
Consultant Pathologist
Department of Pathology

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 1 ♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Tubular dilatation: (Minimal , Focus)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 2♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Area)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Minimal , Focus)

Adrenals

Diffuse cellular vacuolation - zona fasciculata: (Moderate)

Pituitary

Cyst(s) in pars anterior

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 2♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids : (One only); Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 3♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Focus)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Minimal , Foci)

Thyroids

Follicular cell hypertrophy: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 3♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 4♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Increased numbers of alveolar macrophages: (Minimal , Foci)

Heart

Myocarditis: (Minimal , Area)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Moderate)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Pituitary

Focal vacuolation - pars distalis

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 4♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys;
Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids;
Adrenals; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon;
Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11**(Pathology - continued)**

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 5♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: red staining around right eye, top teeth crooked Week 4 to Week 14.

MACROSCOPIC FINDINGS**Incisors**

Maloccluded: (Upper)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Focus)

Heart

Mononuclear cells between myocardial fibres: (Minimal , Foci)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Medullary cyst

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 5♂ - continued

MICROSCOPIC FINDINGS - continued

Adrenals

Diffuse cellular vacuolation - zona fasciculata: (Moderate)

Pituitary

Focal vacuolation - pars distalis

The following tissues were considered normal:

Trachea; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids : (One only); Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11**(Pathology - continued)**

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 6♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss left side of abdomen.

MACROSCOPIC FINDINGS**Skin Alopecia**

Left abdominal region: (Patchy)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocarditis: (Moderate , Foci)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Minimal , Foci)

Thyroids

Follicular cell hypertrophy: (Minimal)

Pituitary

Cyst(s) in pars anterior

Focal vacuolation - pars distalis

Sciatic Nerve

Degenerate fibres: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 6♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Parathyroids; Adrenals; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 7♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Periportal inflammatory cells: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Thyroids

Follicular cell hypertrophy: (Minimal)

Ectopic thymus

Pituitary

Focal vacuolation - pars distalis

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 7♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Adrenals; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 8♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Subpleural aggregation of alveolar macrophages

Heart

Myocarditis: (Minimal , Foci)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Moderate , Foci)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 8♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 9♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Minimal , Foci)

Pituitary

Focal vacuolation - pars distalis

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids : (One only); Adrenals; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 10♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocarditis: (Moderate , Foci)

Spleen

Haemosiderosis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Urinary Bladder

Subepithelial inflammatory cells: (Minimal , Focus)

Prostate

Interstitial inflammation: (Minimal , Foci)

Thyroids

Follicular cell hypertrophy: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 10♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Kidneys; Seminal Vesicles; Epididymides; Testes; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 11♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

The following tissues were considered normal:

Lungs; Liver (ORO stain); Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 12♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss left shoulder Week 3 to 9. Brown staining right forelimb Week 4 to 14. Brown staining left forelimb Week 11 to 14.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodules, near to limiting ridge: (Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Foci)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Pelvic transitional cell hyperplasia: (Minimal , Foci)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 13♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 14♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodules, near to limiting ridge: (Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Generalised fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 15♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 16♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 17♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 18♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Subpleural aggregation of alveolar macrophages

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 19♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: scabs right forelimb Week 10 to 14.

MACROSCOPIC FINDINGS

Skin Scabs

Right forelimb: (Two) 3mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Skin

Scab: (Right forelimb)

Epidermal hyperplasia: (Moderate) (Right forelimb)

Dermal inflammation: (Marked) (Right forelimb)

The following tissues were considered normal:

Lungs; Liver (ORO stain); Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 20♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 21♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 13.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Interstitial inflammatory cells: (Minimal , Focus)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 22♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Generalised fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Moderate , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 23♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

The following tissues were considered normal:

Lungs; Liver; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 24♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Moderate)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Moderate , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 25♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Focus)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

The following tissues were considered normal:

Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 26♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 27♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Tail

Malaligned

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 28♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 29♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: right eye damaged due to blood sampling in Week 13, eye appeared prominent and dry.

MACROSCOPIC FINDINGS

Fur
Stained - periorbital region/s: (Right , Brown)

Subcutis
Haemorrhage: Right periorbital region

Eyes
Damaged: (Right)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Osteoid deposition: (Minimal , Focus)

Liver
Centrilobular hepatocyte vacuolation: (Minimal)
Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)
Periportal fat deposition: (Minimal)

Eyes
Panophthalmitis

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 29♂ - continued

MICROSCOPIC FINDINGS - continued

Subcutis

Haemorrhage and inflammation-periorbital region
Scab and epidermal hyperplasia - periorbital region

The following tissues were considered normal:

Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 30♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 31♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Mononuclear cells between myocardial fibres: (Minimal , Foci)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Prostate

Interstitial inflammation: (Minimal , Focus)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 31♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 32♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 7 to 14.

MACROSCOPIC FINDINGS

Incisors

Maloccluded: (Upper)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Thyroids

Ectopic thymus

Adrenals

Diffuse cellular vacuolation - zona fasciculata: (Minimal)

Pituitary

Focal vacuolation - pars distalis

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 32♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver (ORO stain); Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Parathyroids; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 33♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Moderate)
Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 34♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Haemosiderosis: (Minimal)

Liver

Parenchymal inflammatory cells: (Minimal , Foci)

Hepatocyte necrosis: (Minimal , Foci)

Kidneys

Dilatation of the renal pelvis: (Minimal , Unilateral)

Prostate

Interstitial inflammation: (Minimal , Foci)

Adrenals

Diffuse cellular vacuolation - zona fasciculata: (Minimal)

Pituitary

Focal vacuolation - pars distalis

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 34♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver (ORO stain); Pancreas; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 35♂ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 11 to 14.

MACROSCOPIC FINDINGS

Incisors

Maloccluded: (Upper)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Thyroids

Follicular cell hypertrophy: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 35♂ - continued

MICROSCOPIC FINDINGS - continued

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 36♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Focus)

Heart

Mononuclear cells between myocardial fibres: (Minimal , Focus)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Focus)

Thyroids

Follicular cell hypertrophy: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 36♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 37♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocarditis: (Moderate , Foci)
Myocardial fibrosis: (Moderate , Area)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Adrenals

Diffuse cellular vacuolation - zona fasciculata: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 37♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas;
Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids;
Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon;
Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 38♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Testes

Almost total depletion of germ cells leaving only Sertoli cells: (Minimal ,
Bilateral)

Thyroids

Ectopic thymus

Pituitary

Cyst(s) in pars anterior

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 38♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Parathyroids; Adrenals; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 39♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Increased numbers of alveolar macrophages: (Minimal , Focus)

Heart

Myocardial fibrosis: (Minimal , Focus)

Prostate

Interstitial inflammation: (Minimal , Foci)

Thyroids

Follicular cell hypertrophy: (Minimal)

Pituitary

Focal vacuolation - pars distalis

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 39♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver; Liver (ORO stain); Pancreas; Kidneys; Urinary Bladder; Seminal Vesicles; Epididymides; Testes; Parathyroids; Adrenals; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 40♂ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Moderate , Area)

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Centrilobular hepatocyte hypertrophy: (Minimal)

Hepatocyte necrosis: (Minimal , Focus)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Thyroids

Follicular cell hypertrophy: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 40♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 41 ♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss middle of back Week 13 to 14. Right eye damaged during blood sampling Week 13.

MACROSCOPIC FINDINGS

Fur

Stained - periorbital region/s: (Right , Brown)

Skin Alopecia

Dorsal thoracic region: (Minimal)

Subcutis

Haemorrhage: Right periorbital region

Eyes

Damaged: (Right)

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical

Increased cellularity - generalised
Sinusoidal congestion

Spleen

Haemosiderosis: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 41♀ - continued

MICROSCOPIC FINDINGS - continued

Liver

Extramedullary haemopoiesis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

Eyes

Panophthalmitis: (Unilateral) (Right-inflammation extending into periorbital tissues)

Subcutis

Haemorrhage and inflammation-periorbital region

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 42♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Area)

Spleen

Extramedullary haemopoiesis: (Minimal)
Haemosiderosis: (Minimal)

Liver

Parenchymal inflammatory cells: (Minimal , Focus)
Centrilobular hepatocyte hypertrophy: (Minimal)
Hepatocyte necrosis: (Minimal , Focus)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal , Focal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 42♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 43 ♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss right forelimb Week 5 to 14.

MACROSCOPIC FINDINGS

Skin Alopecia

Forelimb: (Right , Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Extramedullary haemopoiesis: (Minimal)

Haemosiderosis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 44♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Focus)
Mononuclear cells between myocardial fibres: (Minimal , Focus)

Spleen

Haemosiderosis: (Minimal)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)
Dystrophic mineralisation: (Minimal , Foci)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 44♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas;
Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary;
Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone
Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 45♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 14.

MACROSCOPIC FINDINGS

Incisors

Maloccluded: (Upper)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial degeneration: (Minimal , Foci)

Spleen

Extramedullary haemopoiesis: (Minimal)

Haemosiderosis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 46♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Area)

Spleen

Haemosiderosis: (Moderate)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 46♀ - continued

MICROSCOPIC FINDINGS - continued

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 47♀ (Intercurrent)

CLINICAL FINDINGS

Found dead after blood sampling in Week 5.

MACROSCOPIC FINDINGS

Found dead, rat smelt of ether

Lungs

Congested: (Minimal , Patchy)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Vascular congestion: (Marked)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Factors Contributory To Death

Accident: (Post anaesthetic)

The following tissues were considered normal:

Trachea; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 48♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 13 to 14.

MACROSCOPIC FINDINGS

Incisors

Maloccluded: (Upper)

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

Kidneys

Increased pelvic dilatation: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Area)

Spleen

Haemosiderosis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Focal)

Dilatation of the renal pelvis: (Minimal , Unilateral)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 48♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 49♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MACROSCOPIC FINDINGS

Kidneys

Increased pelvic dilatation: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Focus)

Spleen

Haemosiderosis: (Minimal)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dilatation of the renal pelvis: (Minimal , Unilateral)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 49♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 50♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: brown stains on neck Week 5 to 14.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Extramedullary haemopoiesis: (Minimal)
Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric;
Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids : (One only);
Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum;
Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 51 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal , Foci)

Uterus

Luminal dilatation: (Moderate)

The following tissues were considered normal:

Lungs; Liver

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 52♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Uterus

Luminal dilatation: (Moderate)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 53 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal , Foci)

The following tissues were considered normal:

Lungs; Liver

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 54♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)
Periportal fat deposition: (Moderate)

The following tissues were considered normal:

Lungs; Liver; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 55♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)
Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Generalised fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 56♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 57♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Liver; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 58♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Periportal hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 59♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 60♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss both forelimbs Week 5 to 14.

MACROSCOPIC FINDINGS

Skin Alopecia

Forelimb: (Minimal)

Kidneys

Increased pelvic dilatation: (Right , Minimal)

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dilatation of the renal pelvis: (Minimal , Unilateral)

Uterus

Luminal dilatation: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 60♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 61 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Moderate)

Uterus

Luminal dilatation: (Moderate , Focal)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 62♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Kidneys

Increased pelvic dilatation: (Minimal)

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Periportal hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dilatation of the renal pelvis: (Minimal , Unilateral)

Uterus

Luminal dilatation: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 62♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 63♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Periportal hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Uterus

Luminal dilatation: (Moderate)

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 64♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 7 to 14. Red nasal staining Week 7 to 11.

MACROSCOPIC FINDINGS

Incisors

Maloccluded: (Upper)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Osteoid deposition: (Minimal , Focus)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal , Foci)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 65♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodules, near to limiting ridge: (Three) 1mm

Kidneys

Increased pelvic dilatation: (Minimal)

Cortical depression/s: (A few) 1mm

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

Kidneys

Pyelitis: (Severe)

Cortical tubules - basophilic: (Minimal , Foci)

Dystrophic mineralisation: (Moderate , Foci)

Interstitial inflammatory cells: (Severe) (Below pelvic and papillary urothelium)

Pelvic transitional cell hyperplasia: (Severe)

Peripelvic lymphoid aggregations: (Marked)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 65♀ - continued

MICROSCOPIC FINDINGS - continued

Uterus

Luminal dilatation: (Moderate)

The following tissues were considered normal:

Lungs; Liver; Stomach : (W.N.L.)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 66♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: hair loss both forelimbs Week 11 to 13.

MACROSCOPIC FINDINGS

Kidneys

Mass: (Right) 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Pyelitis: (Moderate)

Malignant lymphoma

Lymphoreticular Tumour

Malignant lymphoma: (NEOPLASTIC , MALIGNANT , PRIMARY)

The following tissues were considered normal:

Lungs; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric

Pathologist: S.M.Harling

APPENDIX 11**(Pathology - continued)**

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 67♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: brown staining head Week 13 to 14.

MACROSCOPIC FINDINGS**Fur**

Stained - cranial region: (Minimal , Brown)

Stomach Antrum Mucosa

White nodules, near to limiting ridge: (Two) 1mm

Adrenals

Enlarged: 0.1029g

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Uterus

Luminal dilatation: (Moderate)

Endometrial polypoid hyperplasia: (Minimal , Focus)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 67♀ - continued

MICROSCOPIC FINDINGS - continued

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Kidneys; Adrenals : (W.N.L.)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 68♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 69♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Periportal hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dilatation of the renal pelvis: (Minimal , Unilateral)

The following tissues were considered normal:

Lungs

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 70 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver (ORO stain)

Periportal fat deposition: (Minimal)

The following tissues were considered normal:

Lungs; Liver; Kidneys

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 71 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal , Area)

Spleen

Haemosiderosis: (Moderate)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Uterus

Luminal dilatation: (Minimal , Focal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 71♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 72 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Pneumonitis: (Minimal , Foci)

Spleen

Extramedullary haemopoiesis: (Minimal)

Haemosiderosis: (Minimal)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 72♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas;
Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary;
Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone
Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 73 ♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodules, near to limiting ridge: (Three) 1mm

Kidneys

Cortical depression/s: (Right , One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Mononuclear cells between myocardial fibres: (Minimal , Focus)

Spleen

Haemosiderosis: (Moderate)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Moderate , Area , Unilateral)

Dystrophic mineralisation: (Minimal , Foci)

Interstitial inflammatory cells: (Moderate , Area , Unilateral)

Medullary cyst

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 73♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 74♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Haemosiderosis: (Minimal)

Liver

Extramedullary haemopoiesis: (Minimal , Foci)

Liver (ORO stain)

Hepatocyte fat - focal: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)

Dystrophic mineralisation: (Moderate , Foci)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Uterus; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 75♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: badly groomed Week 13 to 14.

MACROSCOPIC FINDINGS

Fur

Badly groomed - dorsum

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

Kidneys

Cortical depression/s: (A few) 1mm
Increased pelvic dilatation: (Moderate)

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Moderate , Area)

Spleen

Haemosiderosis: (Moderate)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 75♀ - continued

MICROSCOPIC FINDINGS - continued

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)
Dystrophic mineralisation: (Moderate , Foci)
Dilatation of the renal pelvis: (Moderate , Bilateral)
Cystic cortical tubules: (Minimal , Foci , Unilateral)

Uterus

Luminal dilatation: (Moderate)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Trachea; Lungs; Aorta; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver;
Pancreas; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals;
Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone
Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11**(Pathology - continued)**

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 76♀ (Terminal)

CLINICAL FINDINGS

Hunched posture Week 3 to 10, red nasal stains Week 3 to 5. Unsteady gait Week 3 to 6, Week 8 to 10. Piloerection Week 3 to 5. Appeared pale Week 8 to 10.

MACROSCOPIC FINDINGS**Stomach Antrum Mucosa**

White nodules, near to limiting ridge: (Two) 1mm

Uterus

Fluid distension: (Minimal)

Skeletal Muscle

Hindlimbs atrophied

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Haemosiderosis: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Cortical tubules - basophilic: (Minimal , Foci)
Dystrophic mineralisation: (Moderate , Foci)
Pelvic urolithiasis

Uterus

Luminal dilatation: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 76♀ - continued

MICROSCOPIC FINDINGS - continued

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

Skeletal Muscle

Myofibre atrophy: (Moderate , Area)

Myofibre replacement by adipose tissue: (Marked , Area)

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve; Spinal Cord : (W.N.L.) (Cervical Thoracic And Lumbar Sections)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 77♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: bottom teeth pale Week 4 to 14.

MACROSCOPIC FINDINGS

Incisors

Pale: (Lower)

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Subpleural aggregation of alveolar macrophages

Spleen

Extramedullary haemopoiesis: (Minimal)

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte hypertrophy: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal)

Uterus

Luminal dilatation: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 77♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11**(Pathology - continued)**

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 78♀ (Terminal)

CLINICAL FINDINGS

Incidental findings: red stains around left eye Week 2 to 3. Top teeth crooked Week 2 to 14. Red stains left ear, brown stains head Week 4 to 14.

MACROSCOPIC FINDINGS**Fur**

Stained - cranial region: (Minimal , Brown)

Incisors

Maloccluded: (Upper)

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Subpleural aggregation of alveolar macrophages
Osteoid deposition: (Minimal , Focus)

Spleen

Extramedullary haemopoiesis: (Minimal)
Haemosiderosis: (Moderate)

Liver (ORO stain)

Periportal fat deposition: (Trace)

Kidneys

Cortical tubules - basophilic: (Minimal , Focus)
Interstitial inflammatory cells: (Minimal , Focus)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 78♀ - continued

MICROSCOPIC FINDINGS - continued

Uterus

Luminal dilatation: (Moderate)

The following tissues were considered normal:

Trachea; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Liver; Pancreas; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 79♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Extramedullary haemopoiesis: (Minimal)
Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Uterus

Luminal dilatation: (Moderate)

Stomach

Ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 79♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Kidneys; Urinary Bladder; Cervix; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 80♀ (Terminal)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Spleen

Haemosiderosis: (Minimal)

Liver

Centrilobular hepatocyte vacuolation: (Minimal)

Liver (ORO stain)

Periportal fat deposition: (Minimal)

Kidneys

Dystrophic mineralisation: (Minimal)

Cervix

Squamous epithelial hyperplasia
Squamous epithelial hyperkeratosis

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 80♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Pancreas; Urinary Bladder; Uterus; Ovaries; Thyroids; Parathyroids : (One only); Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Bone Marrow/sternum; Brain; Sciatic Nerve

Pathologist: S.M.Harling

N-(n-butyl) thiophosphoric triamide (NBPT)
TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS
INCORPORATING A NEUROTOXICITY SCREEN

Volume 3

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Report issued 14 July 1997



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APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 81♂ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 82♂ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve : (No Nerve Fibres On Longitudinal Section)

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 83♂ (Interim)

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 84♂ (Interim)

CLINICAL FINDINGS

Incidental findings: end of tail swollen Week 8 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 85♂ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 86♂

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 14.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 87♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 88♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 89♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 90♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 91♂

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 14. Red staining around eyes Week 10 to 13.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 92♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 93♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT

Dosage Level: 1000 ppm

Rat No/Sex: 94♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 95♂

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 96♂ (Interim)

CLINICAL FINDINGS

Incidental findings: top teeth crooked, muzzle swollen, red staining around eyes, respiratory noises Week 10 to 14. Placed in solid bottom cage with a food cup due to condition of teeth Week 10 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 97♂ (Interim)

CLINICAL FINDINGS

Incidental findings: red staining around right eye, top teeth crooked Week 4 to 14. Red staining around left eye Week 10 to 13.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 98♂ (Interim)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT

Dosage Level: 5000 ppm

Rat No/Sex: 99♂ (Interim)

CLINICAL FINDINGS

Incidental findings: hair loss both forepaws Week 10 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 100♂ (Interim)

CLINICAL FINDINGS

Piloerection Week 9 to 14.

Incidental findings: top teeth crooked, muzzle swollen, red staining around eyes Week 9 to 14. Placed in solid bottom cage with a food cup due to condition of teeth Week 11 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT

Dosage Level: Control

Rat No/Sex: 101♀ (Interim)

CLINICAL FINDINGS

Incidental findings: brown nasal staining Week 11 to 12.

MICROSCOPIC FINDINGS

The following observations were noted:

Tibial Nerve

(Longitudinal section - missing)

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 102♀ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 103♀ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: Control
Rat No/Sex: 104♀ (Interim)

CLINICAL FINDINGS

No findings of ill health or behavioural change noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT

Dosage Level: Control

Rat No/Sex: 105♀ (Interim)

CLINICAL FINDINGS

Incidental findings: hair loss neck, both forelimbs Week 5 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 106♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 107♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 108♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 109♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 200 ppm
Rat No/Sex: 110♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 111 ♀

CLINICAL FINDINGS

Incidental findings: top teeth crooked Week 10 to 14.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 112♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 113♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 114♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 1000 ppm
Rat No/Sex: 115♀

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 116♀ (Interim)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT

Dosage Level: 5000 ppm

Rat No/Sex: 117♀ (Interim)

CLINICAL FINDINGS

Incidental findings: hair loss both forelimbs Week 11 to 14.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 118♀ (Interim)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MICROSCOPIC FINDINGS

The following observations were noted:

Tibial Nerve
(Longitudinal section missing)

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 119♀ (Interim)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

APPENDIX 11

(Pathology - continued)

Compound: NBPT
Dosage Level: 5000 ppm
Rat No/Sex: 120♀ (Interim)

CLINICAL FINDINGS

No findings of ill health, behavioural change or reaction to treatment noted.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Forebrain/cerebrum; Midbrain; Cerebellum And Pons; Medulla Oblongata; Spinal Cord (C3-6); Spinal Cord (L1-4); Gasserian Ganglia; Dorsal Root Ganglion (C); Dorsal Root Ganglion (L); Dorsal Root Fibres (C); Dorsal Root Fibres (L); Ventral Root Fibres (C); Ventral Root Fibres (L); Sciatic Nerve (Sciatic Notch); Sciatic Nerve (Mid-thigh); Sural Nerve; Tibial Nerve

Pathologist: S.M.Harling

**N-(n-BUTYL) THIOPHOSPHORIC TRIAMIDE
FORMULATION ANALYSIS**

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Andrew Burgess.



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INTRODUCTION

This report details the results obtained for:

The concentrations of N-(n-butyl) thiophosphoric triamide in test diet formulations¹ analysed during the study.

The purity of technical grade N-(n-butyl) thiophosphoric triamide.

The formulations for this study were prepared as a blended admixture of N-(n-butyl) thiophosphoric triamide (batch 8124-165) in rodent diet formulations¹ by Pharmacy personnel at Huntingdon Life Sciences Ltd.

The validation of the analytical procedure, homogeneity and stability of N-(n-butyl) thiophosphoric triamide in rodent diet formulations¹ were determined for concentrations of 100 ppm and 20000 ppm during an earlier study. The experimental details and the analytical results obtained were presented in Huntingdon Life Sciences report IMF 6/961820.

¹ SDS Rat and Mouse No 1 modified maintenance diet

EXPERIMENTAL PROCEDURE

CONCENTRATIONS IN TEST DIET FORMULATIONS

At specified intervals (Weeks 1 and 11) during the study, freshly-prepared test diet formulations were sampled (200 g) from the turbula mixer drum by Pharmacy personnel and submitted for analysis. Each diet sample was sub-sampled (10 g) in duplicate and analysed in accordance with Huntingdon Life Sciences analytical procedure IMF/FA/M112/95.

PROCEDURAL RECOVERIES

At each occasion, the accuracy and precision of the analytical procedure was verified for the range of inclusion levels being examined by analysing a minimum of three procedural recoveries concurrently with test formulations. Procedural recoveries were prepared by fortifying samples (10 g) of control rodent diet with known amounts of N-(n-butyl) thiophosphoric triamide, added either as a solution in methanol (inclusion levels < 2000 ppm) or as solid test material (inclusion levels \geq 2000 ppm). The percentage recovery was calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{Analysed concentration (ppm)}}{\text{Fortified concentration (ppm)}} \times 100$$

DETERMINATION OF THE TEST SUBSTANCE PURITY

The purity of the test substance (technical grade) was determined by HPLC assay prior to initiation and on completion of the neurotoxicity study.

At each occasion triplicate samples (approximately 50 mg) of the technical standard were prepared at a concentration of 12.5 $\mu\text{g/ml}$ in mobile phase and analysed in accordance with the analytical procedure with reference to the analytical grade standard.

RESULTS AND DISCUSSION

The mean concentrations of N-(n-butyl) thiophosphoric triamide in test diet formulations analysed during the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were within 4% of nominal concentrations. Individual analytical results and associated procedural recovery data are detailed in Table 2.

The mean result for the purity of the technical grade N-(n-butyl) thiophosphoric triamide was confirmed as 89.5% - 89.6% for batch 8124-165.

A typical calibration standard graph confirming the linearity of detector response for N-(n-butyl) thiophosphoric triamide over the concentration range 2.5 $\mu\text{g/ml}$ - 12.5 $\mu\text{g/ml}$ is presented in Figure 1. Typical analytical chromatograms are presented in Figures 2 and 3. In Figure 2, the specificity of the HPLC assay was confirmed by the absence of any peaks at the characteristic retention volume for N-(n-butyl) thiophosphoric triamide in the chromatogram obtained for the control sample.

CONCLUSION

The results for the test diet formulations analysed during the study were within 4% of nominal concentrations confirming the accuracy of formulation.

TABLE 1

Summary: mean concentrations of N-(n-butyl) thiophosphoric triamide in test diet formulations

Week of study	Group	Nominal inclusion (ppm)	Mean analysed concentration (ppm)	RME (%)
1	Control	0	ND	—
	2	200	196	−2.0
	3	1000	962	−3.8
	4	5000	4990	−0.2
11	Control	0	ND	—
	2	200	201	+0.5
	3	1000	985	−1.5
	4	5000	4880	−2.4

ND None detected (< 7.5 ppm).

RME Relative mean error, representing the deviation from nominal.

TABLE 2

**Concentrations of N-(n-butyl) thiophosphoric triamide in test diet formulations
(individual values)**

Week of study	Group	Nominal inclusion (ppm)	Analysed concentration (ppm)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean _i
1	Control	0	ND	ND	ND		
	2	200	204	188	196	87.8	
	3	1000	973	951	962	89.6, 86.2	88.5
	4	5000	5000	4970	4990	90.5	
11	Control	0	ND	ND	ND		
	2	200	189, 200	215, 202	201 ²	87.1, 87.2	
	3	1000	1040, 1040	922, 937	985 ²	87.5, 83.0	87.7
	4	5000	4810	4940	4880	90.2	

ND None detected (<7.5 ppm).

¹ Represents the cumulative mean procedural recovery value obtained at analysis.

² Represents the mean of 4 analyses. Re-analysis was undertaken as the duplicate results for the original assay were >±5% of the mean value.

Results were calculated using rounded figures and corrected for the appropriate mean procedural recovery value

TABLE 3

Determination of the purity of technical grade N-(n-butyl) thiophosphoric triamide

Date of analysis	Batch number	Sample	Purity (%)		CV (%)
			Analysis	Mean	
23 May 1996	8124-165	A	90.3	89.5	0.81
		B	88.9		
		C	89.3		
11 September 1996	8124-165	A	90.5	89.6	0.87
		B	89.2		
		C	89.1		

CV Coefficient of variation

FIGURE 1

Typical calibration standard graph
(Week 11)

Method: DKB200: [D1.FORM.IMF.IMF7.AC4997]NBPT_DIET_A.MET;9
 Component: NBPT
 Date: 23-AUG-1996 13:58:51.69
 Linear fit, Origin Treament....Ignore.
 K0: 4.0492E+02 K1: 1.2150E+04
 Coeff. of determination: 0.9998

Standard Sample	Component Area	Component Mass	% Rel. St. Dev.
STD 1	29874	2.4379	
STD 2	60170	4.8759	
STD 3	88344	7.3138	
STD 4	119775	9.7518	
STD 5	148181	12.190	

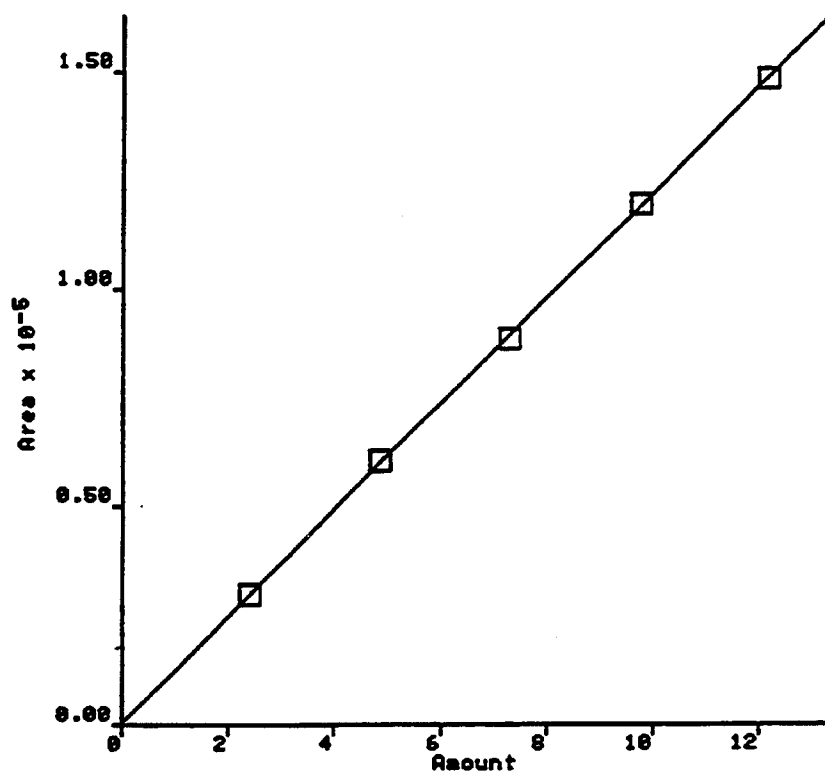
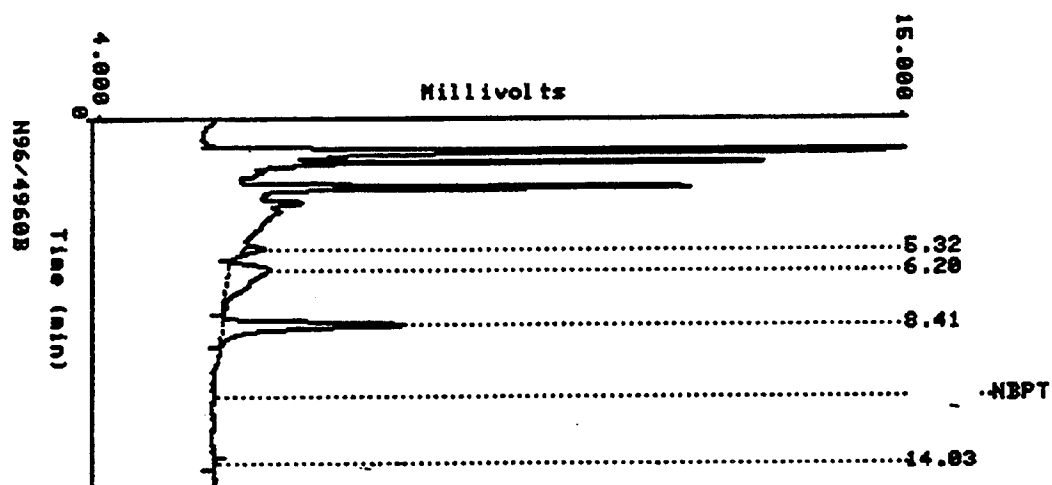


FIGURE 2

Typical sample chromatograms
(Week 11)

Group 1, Control (10 g/200 ml)



Group 2, 200 ppm (10 g/200 ml)

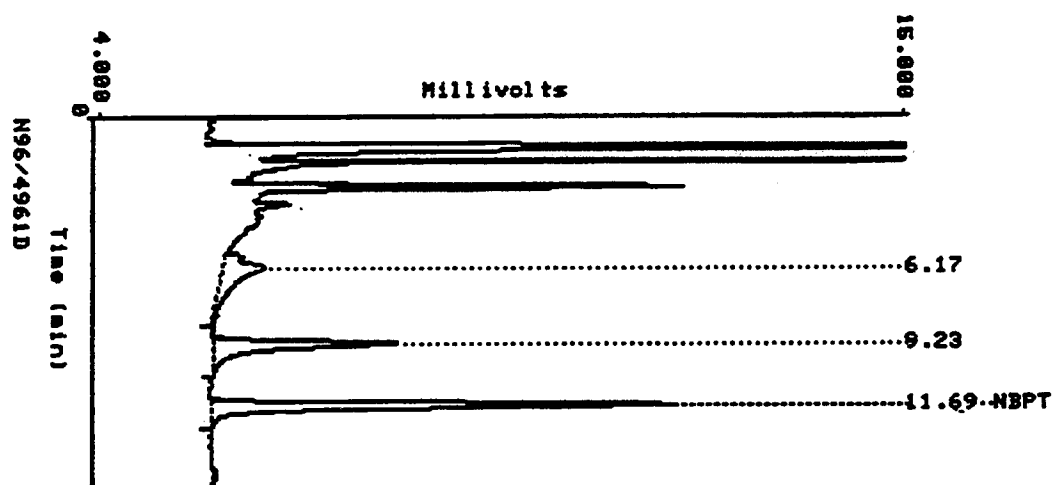
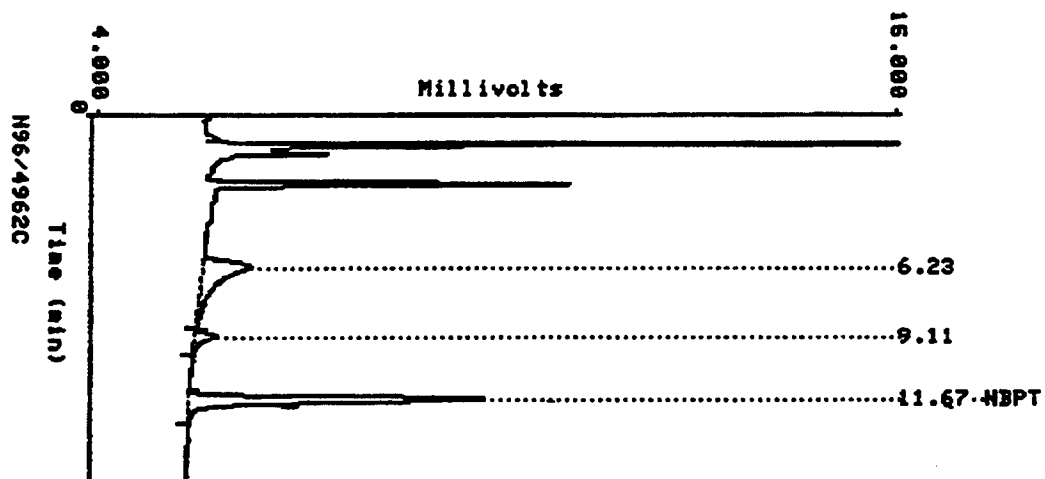


FIGURE 2

(continued)

Group 3, 1000 ppm (10 g/1600 ml)



Group 4, 5000 ppm (10 g/8000 ml)

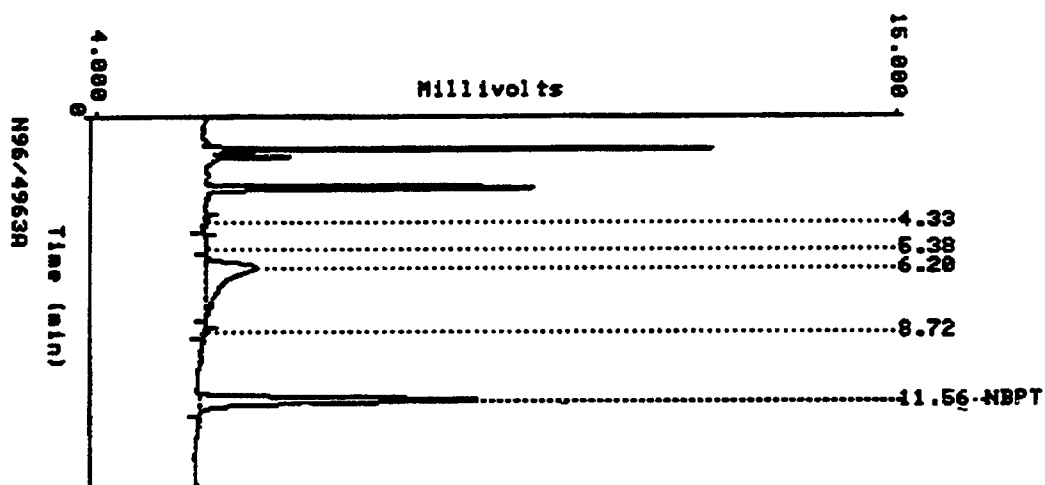
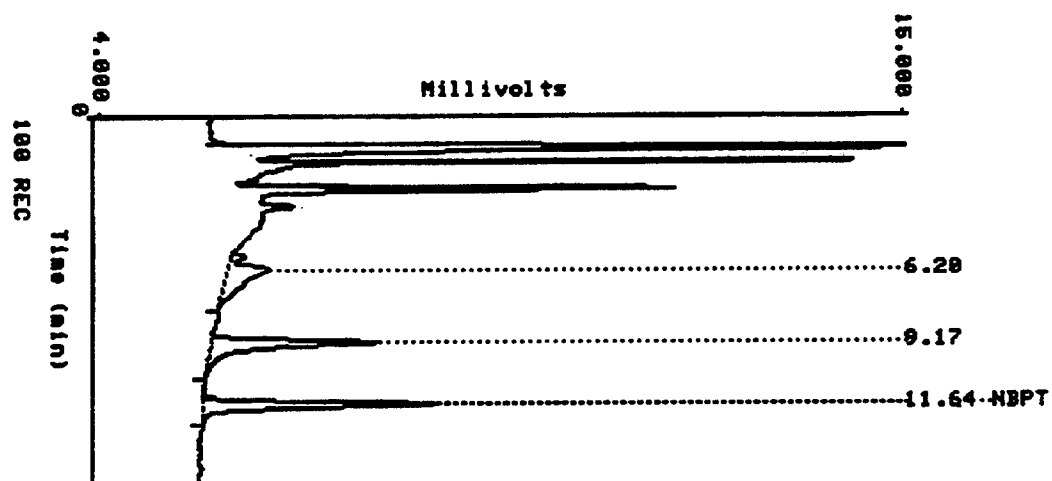


FIGURE 3

Typical procedural recovery chromatograms
(Week 11)

100 ppm, 87.1%



1000 ppm, 87.5%

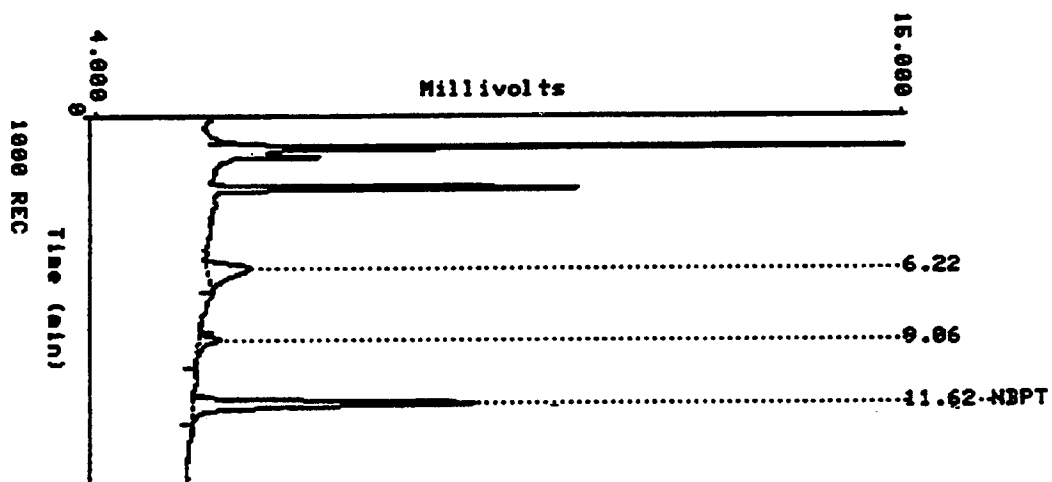


FIGURE 3

(continued)

5000 ppm, 90.2%

